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I, CASSANDRA RICHARDS, ACTING TEAM LEADER EXAMINATION SUPPORT & SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. PQ 2967 for a patent by UNISEARCH LIMITED filed on 20 September 1999.

WITNESS my hand this
Twentieth day of October 2000

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ORIGINAL

AUSTRALIA

Patents Act 1990

PROVISIONAL SPECIFICATION FOR THE INVENTION ENTITLED:

A Cell-Membrane Impermeable Trivalent Organoarsenical Derivative and Use Thereof

Name and Address of Applicant: Unisearch Limited, an Australian company, ACN 000 263 025, of The University of New South Wales, Sydney, NSW, 2052, AUSTRALIA

Inventor(s) Name(s): Philip John Hogg and Neil Donoghue

This invention is best described in the following statement:

A Cell-Membrane Impermeable Trivalent Organoarsenical Derivative and Use Thereof

Technical Field

The present invention relates to cell-membrane impermeable compounds having the ability to inhibit redox active proteins and to methods for their synthesis. In particular, the invention relates to cell-membrane impermeable trivalent organoarsenical compounds and to methods for their synthesis. The invention also relates to pharmaceutical compositions and to methods of treatment of inflammatory disorders, autoimmune diseases, blood vessel diseases, thrombosis, viral infections, and haematological and solid tumours.

Background of the Invention

Some secreted proteins undergo redox reactions, that is transfer or shuffling of hydrogens and electrons between amino acids (1, 2). The amino acid most often involved is cysteine, the redox reaction involving in particular the cysteine thiol. Redox changes in cysteine residues can lead to net reduction, net formation or net interchange of disulfide bonds.

For example, protein disulfide isomerase is a cell-surface redox active enzyme that has been implicated in reduction of the disulfide-linked diphtheria toxin heterodimer (3, 4), cell surface events which trigger entry of the human immunodeficiency virus into lymphoid cells (5), shedding of the human thyrotropin receptor ectodomain (6), as a cell surface recognition/adhesion molecule during neuronal differentiation of the retina (7), and transnitrosation and intracellular transfer of nitric oxide (8). Protein disulfide isomerase is also on the external surface of the platelet plasma membrane and is involved in platelet aggregation and secretion (9, 10). Increase or decrease in protein disulfide isomerase on the surface of HT1080 human fibrosarcoma cells is associated with increase or decrease in cell surface protein thiols (1) and cell surface protein disulfide isomerase has been implicated in the increase in surface protein thiol content of human lymphocytes following mitogen activation (2, 11). Also, human fibrosarcoma cells secrete a redox active enzyme called plasmin reductase (12). Plasmin reductase reduces two or more disulfide bonds in the serine proteinase, plasmin, which triggers formation of the tumour angiogenesis inhibitor, angiostatin (13). In addition, endothelial cells secrete a disulfide bond reductase which reduces the average multimer size and haemostatic activity of the blood protein, von Willebrand factor, and indicate that certain secreted protein thiol(s)/disulfide(s) are under redox control.

It is apparent that the function of some extracellular proteins is controlled by the redox state of two or more of their cysteine residues. Moreover, increased cellular activity correlates with increased cell surface redox events (1, 2, 11 and references therein). Furthermore, cells can control the redox state of extracellular protein thiols/disulfides through secretion of reductases/isomerases (1). A compound which inhibits secreted reductases/isomerases and/or redox active cell surface proteins has the potential to change the properties of the cell, in particular rapidly proliferating cells. For instance, such a compound may preferentially target the proliferating endothelial and transformed cells in solid tumours.

The present invention provides compounds wherein a chemical moiety having the ability to inhibit redox active proteins is linked to a cell membrane impermeable pendant group. That is, the present invention provides compounds in which an ionic pendant is linked, with or without the incorporation of a spacer group, to a chemical moiety having the ability to inhibit redox active proteins.

Glutathione (GSH) is a tripeptide that is constitutively secreted by mammalian cells but is not taken up by these cells (15) and is an example of the cell membrane impermeable pendant group. In a specific embodiment, the present invention provides compounds wherein a chemical moiety having the ability to inhibit redox active proteins is linked to glutathione through the free thiol. In a further embodiment, the present invention provides the attachment of a trivalent organoarsenical to GSH through the free thiol to produce a novel dithiol-reactive cell membrane-impermeable compound.

Disclosure of the Invention

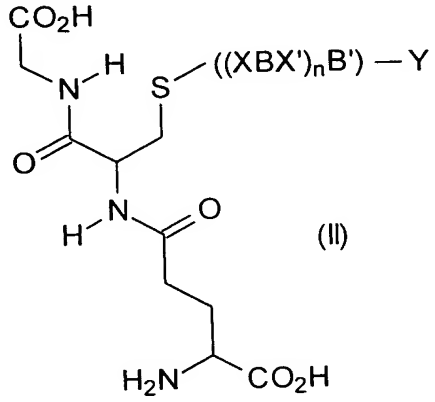
According to a first embodiment of the invention, there is provided a compound according to the Formula I:

$$A\text{-}(XBX')_nB'\text{-}Y, \quad (I)$$

wherein A comprises any suitable multiply charged, at neutral pH, cell-membrane impermeable pendant group, and wherein $(XBX')_nB'$ comprises any suitable linker and/or spacer group and wherein Y comprises a chemical moiety having the ability to inhibit redox active proteins.

Typically, A is selected from the group consisting of glutathione, cysteinylglycine, cysteine, aspartic acid, glutamic acid, lysine, arginine, 3,5-diiodo-L-tyrosine and 5-aminoisophthalic acid; wherein the sulfur atom of each glutathione, cysteinylglycine and cysteine may form a sulfide or be optionally oxidised to form a sulfoxide or sulfone.

More typically, A is glutathione and in one form of the invention the compound is as represented in the following Formula II:



(II)

wherein $(XBX')_nB'$ comprises any suitable linker and/or spacer group and wherein Y comprises any chemical moiety having the ability to inhibit redox active proteins.

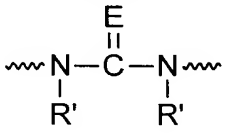
Typically, Y is selected from the group consisting of: arsenoxide, and arsenoxide equivalent.

More typically, Y is an arsenoxide group, as can be represented by –As=O.

Typically, X is selected from -NR-, -S(O)-, -S(O)O-, $-S(O)_2-$, $-S(O)_2O-$, -C(O)-, -C(S)-, -C(O)O-, C(S)O-, -C(S)S-, $-P(O)(R_1)-$, $-P(O)(R_1)O-$ or is absent;

B is selected from C_1-C_{10} alkylene, C_2-C_{10} alkenylene, C_2-C_{10} alkynylene, C_3-C_{10} cycloalkylene, C_5-C_{10} cycloalkenylene, C_3-C_{10} heterocycloalkylene, C_5-C_{10} heterocycloalkenylene, C_6-C_{12} arylene, heteroarylene or C_2-C_{10} acyl;

X' is selected from –O-, -S-, -NR-, -S-S-, -S(O)-, -OS(O)-, -OS(O)O-, -$S(O)_2$-, -$OS(O)_2$, -$OS(O)_2O$-, -P(O)(R_1)-, -OP(O)(R_1)-, -OP(O)(R_1)O-, -OP(O)(R_1)OP(O)(R_1)O-, -Se-,



or is absent; wherein E is O, S, Se, NR or $N(R)_2^+$;

n is 0, 1 or 2; and

B' is C_1-C_{10} alkylene, C_2-C_{10} alkenylene, C_2-C_{10} alkynylene, C_3-C_{10} cycloalkylene, C_5-C_{10} cycloalkenylene, C_3-C_{10} heterocycloalkylene, C_5-C_{10} heterocycloalkenylene, C_6-C_{12} arylene, heteroarylene or is absent; and wherein

each R is independently selected from hydrogen, C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_3-C_{10} heterocycloalkyl, C_5-C_{10} heterocycloalkenyl, C_6-C_{12} aryl, heteroaryl, OR_2 or C_2-C_{10} acyl;

R' is the same as R or two R' may be taken together with the nitrogen atoms to which they are attached to form a 5 or 6 membered saturated or unsaturated heterocyclic ring;

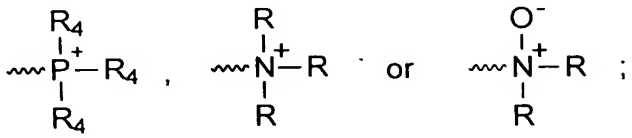
each R_1 is independently selected from hydrogen, C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_3-C_{10} heterocycloalkyl, C_5-C_{10} heterocycloalkenyl, C_6-C_{12} aryl, heteroaryl, halo, OR_2 or $N(R)_2$;

each R_2 is independently selected from hydrogen, C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_3-C_{10} heterocycloalkyl, C_5-C_{10} heterocycloalkenyl, C_6-C_{12} aryl, heteroaryl or -C(O)R_5;

each R_5 is independently selected from hydrogen, C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_3-C_{10} heterocycloalkyl, C_5-C_{10} heterocycloalkenyl, C_6-C_{12} aryl, heteroaryl, C_1-C_{10} alkoxy, C_3-C_{10} alkenyloxy, C_3-C_{10} alkynyloxy, C_3-C_{10} cycloalkyloxy, C_5-C_{10} cycloalkenyloxy, C_3-C_{10} heterocycloalkyloxy, C_5-C_{10} heterocycloalkenyloxy, C_6-C_{12} aryloxy, heteroaryloxy, C_1-C_{10} alkylthio, C_3-C_{10} alkenylthio, C_3-C_{10} alkynylthio, C_3-C_{10} cycloalkylthio, C_5-C_{10} cycloalkenylthio, C_3-C_{10} heterocycloalkylthio, C_5-C_{10} heterocycloalkenylthio, C_6-C_{12} arylthio, heteroarylthio, OH, SH or $N(R)_2$;

wherein each instance of arylene may have substituents A and X or X and Y in a para, meta or ortho relationship, and

wherein each alkylene, alkenylene, alkynylene, cycloalkylene, cycloalkenylene, heterocycloalkylene, heterocycloalkenylene, arylene, heteroarylene and acyl may be independently substituted with hydrogen, C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_3-C_{10} heterocycloalkyl, C_5-C_{10} heterocycloalkenyl, C_6-C_{12} aryl, heteroaryl, halo, OR_{2a}, SR_6, nitro, arsenoxide, -S(O)R_3, -OS(O)R_3, -$S(O)_2R_3$, -$OS(O)_2R_3$, -P(O)R_4R_4, -OP(O)R_4R_4, -$N(R'')_2$, -$NRC(O)(CH_2)_mQ$, -C(O)R_5,



wherein R, R_1 and R_5 are as defined above; and

R_{2a} is selected from hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, -S(O)R_3, -S(O)$_2R_3$, -P(O)$(R_4)_2$, N$(R)_2$ or -C(O)R_5;

each R_3 is independently selected from hydrogen, C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_3-C_{10} heterocycloalkyl, C_5-C_{10} heterocycloalkenyl, C_6-C_{12} aryl, heteroaryl, C_1-C_{10} alkoxy, C_3-C_{10} alkenyloxy, C_3-C_{10} alkynyloxy, C_3-C_{10} cycloalkyloxy, C_5-C_{10} cycloalkenyloxy, C_3-C_{10} heterocycloalkyloxy, C_5-C_{10} heterocycloalkenyloxy, C_6-C_{12} aryloxy, heteroaryloxy, C_1-C_{10} alkylthio, C_3-C_{10} alkenylthio, C_3-C_{10} alkynylthio, C_3-C_{10} cycloalkylthio, C_5-C_{10} cycloalkenylthio, C_3-C_{10} heterocycloalkylthio, C_5-C_{10} heterocycloalkenylthio, C_6-C_{12} arylthio, heteroarylthio or N$(R)_2$;

each R_4 is independently selected from hydrogen, C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_3-C_{10} heterocycloalkyl, C_5-C_{10} heterocycloalkenyl, C_6-C_{12} aryl, heteroaryl, C_1-C_{10} alkoxy, C_3-C_{10} alkenyloxy, C_3-C_{10} alkynyloxy, C_3-C_{10} cycloalkyloxy, C_5-C_{10} cycloalkenyloxy, C_3-C_{10} heterocycloalkyloxy, C_5-C_{10} heterocycloalkenyloxy, C_6-C_{12} aryloxy, heteroaryloxy, C_1-C_{10} alkylthio, C_3-C_{10} alkenylthio, C_3-C_{10} alkynylthio, C_3-C_{10} cycloalkylthio, C_5-C_{10} cycloalkenylthio, C_3-C_{10} heterocycloalkylthio, C_5-C_{10} heterocycloalkenylthio, C_6-C_{12} arylthio, heteroarylthio, halo or N$(R)_2$;

R_6 is selected from C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_3-C_{10} heterocycloalkyl, C_5-C_{10} heterocycloalkenyl, C_6-C_{12} aryl, heteroaryl, C_1-C_{10} alkylthio, C_3-C_{10} alkenylthio, C_3-C_{10} alkynylthio, C_3-C_{10} cycloalkylthio, C_5-C_{10} cycloalkenylthio, C_3-C_{10} heterocycloalkylthio, C_5-C_{10} heterocycloalkenylthio, C_6-C_{12} arylthio, heteroarylthio, -S(O)R_3, -S(O)$_2R_3$ or -C(O)R_5,

R" is the same as R or two R" taken together with the N atom to which they are attached may form a saturated, unsaturated or aromatic heterocyclic ring system;

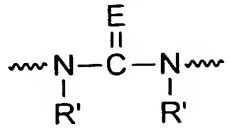
Q is selected from halogen and –OS(O)$_2Q_1$; wherein Q_1 is selected from C_1-C_4 alkyl, C_1-C_4 perfluoroalkyl, phenyl, *p*-methylphenyl; and

m is 1 to 5.

More typically, X is selected from -C(O)-, -C(S)-, -C(O)O-, C(S)O-, -C(S)S- or is absent;

B is selected from C_1-C_5 alkylene, C_2-C_5 alkenylene, C_2-C_5 alkynylene, C_3-C_{10} cycloalkylene, C_5-C_{10} cycloalkenylene, C_6-C_{12} arylene or C_2-C_5 acyl;

X' is selected from –O-, -S-, -NR-, -S-S-, -S(O)-, -S(O)$_2$-, -P(O)(R_1)-, -OP(O)(R_1)-, OP(O)(R_1)O-, -OP(O)(R_1)OP(O)(R_1)O-, -Se-,



or is absent; wherein E is O, S or N$(R)_2^+$;

n is 0, 1 or 2; and

B' is C_1-C_5 alkylene, C_2-C_5 alkenylene, C_2-C_5 alkynylene, C_3-C_{10} cycloalkylene, C_5-C_{10} cycloalkenylene, C_6-C_{12} arylene or is absent; and wherein

each R is independently selected from hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, OR_2 or C_2-C_{10} acyl;

R' is the same as R;

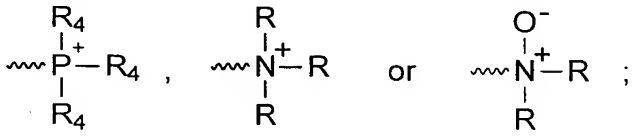
each R_1 is independently selected from hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, halo, OR_2 or $N(R)_2$;

each R_2 is independently selected from hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl or $-C(O)R_5$;

each R_5 is independently selected from hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, C_1-C_5 alkoxy, C_3-C_5 alkenyloxy, C_3-C_5 alkynyloxy, C_3-C_{10} cycloalkyloxy, C_5-C_{10} cycloalkenyloxy, C_6-C_{12} aryloxy, C_1-C_5 alkylthio, C_3-C_5 alkenylthio, C_3-C_5 alkynylthio, C_3-C_{10} cycloalkylthio, C_5-C_{10} cycloalkenylthio, C_6-C_{12} arylthio, OH, SH or $N(R)_2$;

wherein each instance of arylene may have substituents A and X or X and Y in a para, meta or ortho relationship, and

wherein each alkylene, alkenylene, alkynylene, cycloalkylene, cycloalkenylene, arylene, and acyl may be independently substituted with hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, halo, OR_{2a}, SR_6, nitro, arsenoxide, $-S(O)R_3$, $-OS(O)R_3$, $-S(O)_2R_3$, $-OS(O)_2R_3$, $-P(O)R_4R_4$, $-OP(O)R_4R_4$, $-N(R'')_2$, $NRC(O)(CH_2)_mQ$, $-C(O)R_5$,



wherein R, R_1 and R_5 are as defined above; and

R_{2a} selected from hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, $-S(O)R_3$, $-S(O)_2R_3$, $-P(O)(R_4)_2$, $N(R)_2$ or $-C(O)R_5$;

each R_3 is independently selected from hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, C_1-C_5 alkoxy, C_3-C_5 alkenyloxy, C_3-C_5 alkynyloxy, C_3-C_{10} cycloalkyloxy, C_5-C_{10} cycloalkenyloxy, C_6-C_{12} aryloxy, C_1-C_5 alkylthio, C_3-C_5 alkenylthio, C_3-C_5 alkynylthio, C_3-C_{10} cycloalkylthio, C_5-C_{10} cycloalkenylthio, C_6-C_{12} arylthio or $N(R)_2$;

each R_4 is independently selected from hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, C_1-C_5 alkoxy, C_3-C_5 alkenyloxy, C_3-C_5 alkynyloxy, C_3-C_{10} cycloalkyloxy, C_5-C_{10} cycloalkenyloxy, C_6-C_{12} aryloxy, C_1-C_5 alkylthio, C_3-C_5 alkenylthio, C_3-C_5 alkynylthio, C_3-C_5 cycloalkylthio, C_5-C_5 cycloalkenylthio, C_6-C_{12} arylthio, halo or $N(R)_2$;

R_6 is independently selected from C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, C_1-C_5 alkylthio, C_3-C_5 alkenylthio, C_3-C_5 alkynylthio, C_3-C_{10} cycloalkylthio, C_5-C_{10} cycloalkenylthio, C_6-C_{12} arylthio, $-S(O)R_3$, $-S(O)_2R_3$ or $-C(O)R_5$,

R'' is the same as R;

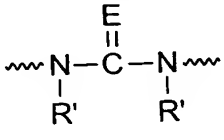
Q is selected from halogen and $-OS(O)_2Q_1$; wherein Q_1 is selected from C_1-C_4 alkyl, C_1-C_4 perfluoroalkyl, phenyl, *p*-methylphenyl; and

m is 1 to 5.

Even more typically, X is absent;

B is selected from C_1-C_5 alkylene, C_6-C_{12} arylene or C_2-C_5 acyl;

X' is selected from –O-, -S-, -NR-, -S-S-, -S(O)-, -$S(O)_2$-, -P(O)(R_1)-, -Se-,



or absent; wherein E is O, S or $N(R)_2^+$;

n is 0, 1 or 2; and

B' is C_1-C_5 alkylene, C_6-C_{12} arylene or is absent; and wherein

each R is independently selected from hydrogen, C_1-C_5 alkyl, C_3-C_{10} cycloalkyl, C_6-C_{12} aryl, OR_2 or C_2-C_5 acyl;

R' is the same as R;

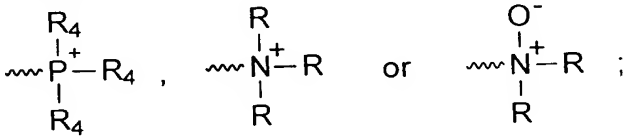
each R_1 is independently selected from hydrogen, C_1-C_5 alkyl, C_3-C_{10} cycloalkyl, C_6-C_{12} aryl, halo, OR_2 or $N(R)_2$;

each R_2 is independently selected from hydrogen, C_1-C_5 alkyl, C_3-C_{10} cycloalkyl, C_6-C_{12} aryl or -C(O)R_5;

each R_5 is independently selected from hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, C_1-C_5 alkoxy, C_3-C_5 alkenyloxy, C_3-C_{10} cycloalkyloxy, C_5-C_{10} cycloalkenyloxy, C_6-C_{12} aryloxy, C_1-C_5 alkylthio, C_3-C_5 alkenylthio, C_3-C_{10} cycloalkylthio, C_5-C_{10} cycloalkenylthio, C_6-C_{12} arylthio, OH, SH or $N(R)_2$;

wherein each instance of arylene may have substituents A and X or X and Y in a para, meta or ortho relationship, and

wherein each alkylene, alkenylene, alkynylene, cycloalkylene, cycloalkenylene, arylene, and acyl may be independently substituted with hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, halo, OR_{2a}, SR_6, nitro, arsenoxide, -S(O)R_3, -OS(O)R_3, -$S(O)_2R_3$, -$OS(O)_2R_3$, -P(O)R_4R_4, -OP(O)R_4R_4, -$N(R'')_2$, -NRC(O)$(CH_2)_m$Q, -C(O)R_5,



wherein R, R_1 and R_5 are as defined above; and

R_{2a} is selected from hydrogen, C_1-C_5 alkyl, C_3-C_{10} cycloalkyl, C_6-C_{12} aryl, -S(O)R_3, -$S(O)_2R_3$, -$P(O)(R_4)_2$ and –C(O)R_5;

each R_3 is independently selected from hydrogen, C_1-C_5 alkyl, C_3-C_{10} cycloalkyl, C_6-C_{12} aryl, C_1-C_5 alkoxy, C_3-C_{10} cycloalkyloxy, C_6-C_{12} aryloxy, C_1-C_5 alkylthio, C_3-C_{10} cycloalkylthio, C_6-C_{12} arylthio or $N(R)_2$;

each R_4 is independently selected from hydrogen, C_1-C_5 alkyl, C_3-C_{10} cycloalkyl, C_6-C_{12} aryl, C_1-C_5 alkoxy, C_3-C_{10} cycloalkyloxy, C_6-C_{12} aryloxy, halo or $N(R)_2$;

R_6 is selected from C_1-C_5 alkyl, C_3-C_{10} cycloalkyl, C_6-C_{12} aryl, C_1-C_5 alkylthio, C_3-C_{10} cycloalkylthio, C_6-C_{12} arylthio, -S(O)R_3, -$S(O)_2R_3$ or –C(O)R_5.

R" is the same as R;

Q is selected from halogen and $-OS(O)_2Q_1$; wherein Q_1 is selected from C_1-C_4 alkyl, C_1-C_4 perfluoroalkyl, phenyl, *p*-methylphenyl; and

m is 1 to 5.

Still more typically, X is absent;

B is selected from C_1-C_5 alkylene, C_6-C_{12} arylene or C_2-C_5 acyl;

X' is selected from –O-, -S-, -NR- or is absent;

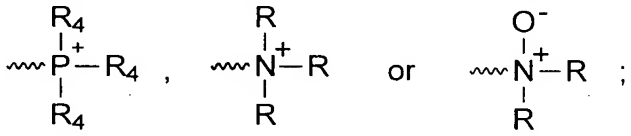
n is 1; and

B' is C_1-C_5 alkylene, C_6-C_{12} arylene or is absent; and

R is selected from hydrogen, C_1-C_5 alkyl, C_6-C_{12} aryl or C_2-C_5 acyl;

wherein each instance of arylene may have substituents A and X or X and Y in a para, meta or ortho relationship, and

wherein each alkylene, arylene, and acyl may be independently substituted with hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, halo, OR_{2a}, SR_6, nitro, arsenoxide, $-S(O)R_3$, $-S(O)_2R_3$, $-P(O)R_4R_4$, $-N(R")_2$, $-NRC(O)(CH_2)_mQ$, $-C(O)R_5$,



wherein each R is independently selected from hydrogen, C_1-C_5 alkyl, C_6-C_{12} aryl or C_2-C_5 acyl;

R_{2a} is selected from hydrogen, C_1-C_5 alkyl, C_6-C_{12} aryl, $-S(O)R_3$, $-S(O)_2R_3$, $-P(O)(R_4)_2$ or $-C(O)R_5$;

each R_3 is independently selected from hydrogen, C_1-C_5 alkyl, C_6-C_{12} aryl, C_1-C_5 alkoxy, C_6-C_{12} aryloxy, C_1-C_5 alkylthio, or C_6-C_{12} arylthio;

each R_4 is independently selected from hydrogen, C_1-C_5 alkyl, C_6-C_{12} aryl, C_1-C_5 alkoxy, C_6-C_{12} aryloxy, C_1-C_5 alkylthio, C_6-C_{12} arylthio, halo or $N(R)_2$;

each R_5 is independently selected from hydrogen, C_1-C_5 alkyl, C_6-C_{12} aryl, C_1-C_5 alkoxy, C_6-C_{12} aryloxy, C_1-C_5 alkylthio, C_6-C_{12} arylthio, OH, SH or $N(R)_2$;

R_6 is selected from C_1-C_5 alkyl, C_6-C_{12} aryl, C_1-C_5 alkylthio, C_6-C_{12} arylthio, $-S(O)R_3$, $-S(O)_2R_3$ or $-C(O)R_5$,

R" is the same as R above;

Q is selected from halogen and $-OS(O)_2Q_1$; wherein Q_1 is selected from C_1-C_4 alkyl, C_1-C_4 perfluoroalkyl, phenyl, *p*-methylphenyl; and

m is 1 to 5.

Yet still more typically, X is absent;

B is C_2-C_5 acyl;

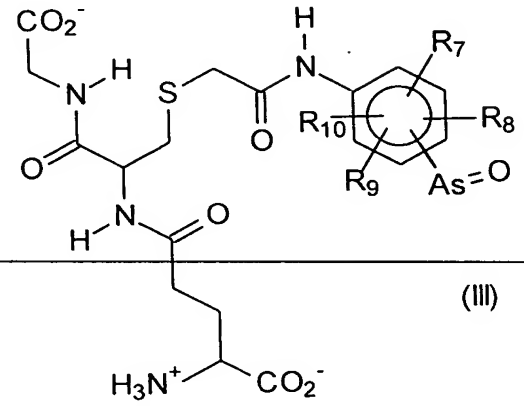
X' is NR;

n is 1;

B' is phenylene; and

R is H;

wherein phenylene may have substituents X and Y in an ortho, meta or para relationship; as exemplified by Formula III:



(III)

and wherein R_7 to R_{10} are independently selected from the group consisting of: hydrogen, C_1-C_5 alkyl, C_6-C_{12} aryl, halogen, hydroxy, amino, nitro, carboxy, C_1-C_5 alkoxy, $-OS(O)_2R_3$ or $-NHC(O)CH_2Q$ wherein Q is halogen, $-OS(O)_2CH_3$, $-OS(O)_2C_6H_5$ or $-OS(O)_2$-*p* tolyl.

More typically, R_7 to R_{10} are independently selected from the group consisting of: hydrogen, halogen, hydroxy, amino, nitro, carboxy, C_1-C_5 alkoxy, methyl, ethyl, iso-propyl, tert-butyl, phenyl and $-NHC(O)CH_2Q$ wherein Q is halogen, $-OS(O)_2CH_3$, $-OS(O)_2C_6H_5$ or $-OS(O)_2$-*p* tolyl.

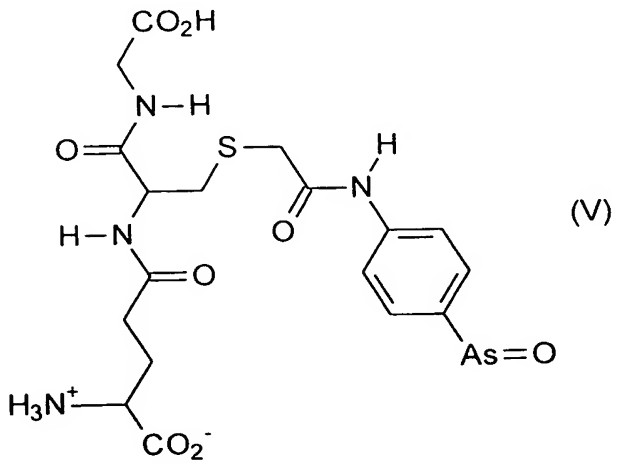
There are also provided by the present invention derivatives of the compounds of Formula III wherein multiple amino-phenylarsenoxide groups are attached to the carbon between the C=O and the sulphur atom of the glutathione pendant. These compounds may be represented in the following Formula IV:

$$GS—C(H)_{3-n}—(J)_n \qquad (IV),$$

wherein GS represents the glutathione moiety and J represents the spacer group $(XBX')_nB'$ and the chemical moiety having the ability to inhibit redox active proteins, and wherein n is 1, 2, or 3.

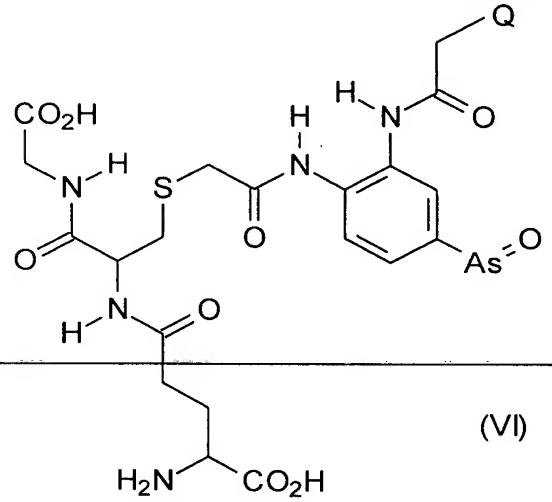
In a preferred form of the invention, the As=O is at the 4–position of the aromatic ring.

More preferably there is provided the compound 4-(*N*-(*S*-glutathionylacetyl)amino)phenylarsenoxide, which can be abbreviated to GSAO, according to Formula V:



(V)

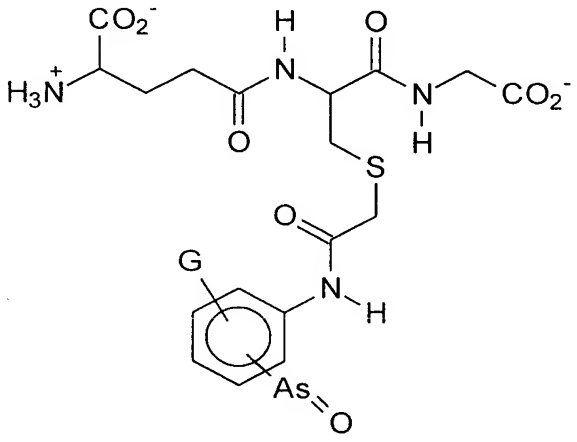
There are also provided by the present invention compounds according to Formula VI:



(VI)

wherein Q is any halogen. For example, the invention provides the compounds 3-(*N*-(fluoroacetyl)amino)-4-(*N*-(*S*-glutathionylacetyl)amino)phenylarsenoxide, which may be abbreviated to GSFAO, 3-(*N*-(chloroacetyl)amino)-4-(*N*-(*S*-glutathionylacetyl)amino)phenylarsenoxide, which may be abbreviated to GSCAO, 3-(*N*-(bromoacetyl)amino)-4-(*N*-(*S*-glutathionylacetyl)amino)phenylarsenoxide, which may be abbreviated to GSBAO, and 3-(*N*-(iodoacetyl)amino)-4-(*N*-(*S*-glutathionylacetyl)amino)phenylarsenoxide, which may be abbreviated to GSIAO.

In another preferred form of the compound of the invention there is provided a compound according to Formula VII:



(VII)

wherein G is selected from the group consisting of: hydrogen, halogen, hydroxy, amino, nitro, carboxy, C_1-C_5 alkoxy, C_1-C_5 alkyl and C_6-C_{12} aryl and -NHC(O)CH_2Q wherein Q is halogen, -$OS(O)_2CH_3$, -$OS(O)_2C_6H_5$ or –$OS(O)_2$-*p* tolyl.

Typically, G is selected from the group consisting of: hydrogen, halogen, hydroxy, amino, nitro, carboxy, C_1-C_5 alkoxy, methyl, ethyl, iso-propyl, tert-butyl, phenyl, and -NHC(O)CH_2Q wherein Q is halogen, -$OS(O)_2CH_3$, -$OS(O)_2C_6H_5$ or –$OS(O)_2$-*p* tolyl.

More typically, in a compound of Formula VII, G is hydroxy, flourine, NH_2, or NO_2.

Typically the activity of the arsenic atom may be modified by the group G, when G and the arsenic atom are in an ortho or para relationship to one another. For example, when G is an electron donating group like OH (ionised to O^- at physiological pH), the arsenic should be deactivated towards dithiols, and so become more selective, only blocking very reactive dithiols. Alternatively, when G is NO_2, electron density would be removed from the arsenic atom, making it more reactive to all dithiols. Selective inhibition of some redox proteins and not others may be achieved by manipulation of G.

According to a second embodiment of the invention there is provided a compound according to any one of Formulae I, II, III, IV, V, VI, or VII wherein the arsenoso (As=O) group is replaced by an arsenoxide equivalent .

An arsenoxide equivalent is any trivalent arsenical that is (a) either hydrolysed to –As=O or $-As(OH)_2$ when dissolved in an aqueous medium (such as cell culture buffers and the fluids contained in the organism being treated), or (b) shows the same affinity towards dithiols as do –As=O and $-As(OH)_2$.

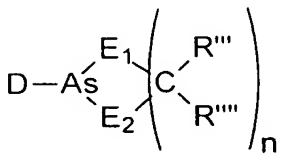
Typically, an arsenoxide equivalent for -As=O will be $-As(Z_1)(Z_2)$. In water $-As(Z_1)(Z_2)$ can hydrolyse to give either $-As(OH)_2$ or -As=O. In other words, -As=O (or its equivalent $As(OH)_2$) can be generated *in situ*, and so the activity characteristic of -As=O will be observed for $-As(Z_1)(Z_2)$, meaning that the equivalent forms of the compounds are expected to exhibit identical or substantially identical activity to the corresponding arsenoxides.

Typically, in the equivalent for -As=O, namely $-As(Z_1)(Z_2)$, Z_1 and Z_2 will be labile groups (i.e. easily displaced under physiological conditions). For example Z_1 and Z_2 may be selected from the group consisting of: OH, OR, SR, SeR, F, Cl, Br, and I wherein R is an alkyl or an aryl group.

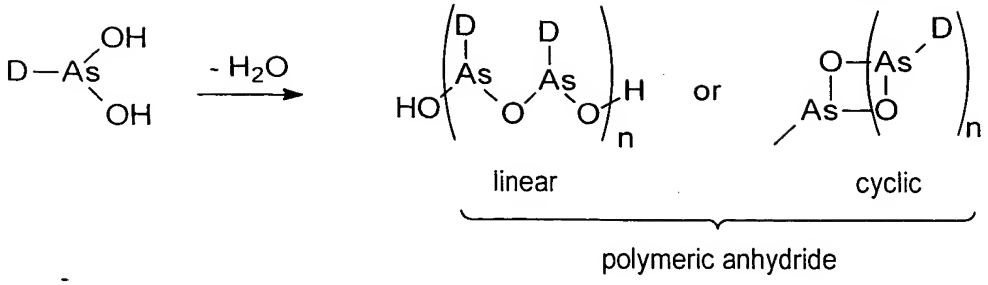
Typically, Z_1 and Z_2, may either be identical or different, and may either be connected or independent from each other (bound only through arsenic).

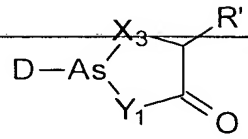
Suitable arsenoxide equivalents for the arsenoso (As=O) group include the following:

$R\text{-}As(Z_1)(Z_2)$ where Z_1 and Z_2 are selected from OH, C_1-C_{10} alkoxy, C_6-C_{10} aryloxy, C_1-C_{10} alkylthio, C_6-C_{10} arylthio, C_1-C_{10} alkylseleno, C_6-C_{10} arylseleno, F, Cl, Br and I;

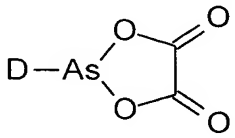


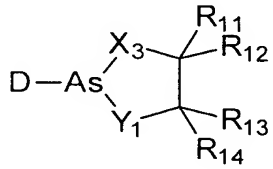
Where $E_1 = E_2 = O$, $E_1 = O$ and $E_2 = S$ or $E_1 = E_2 = S$; R''' and R'''' are independently selected from the group consisting of hydrogen, C_1-C_{10} alkyl, C_6-C_{12} aryl, halogen, C_1-C_{10} alkoxy, C_6-C_{10} aryloxy, hydroxy and carboxy; and n = 1 to 10;



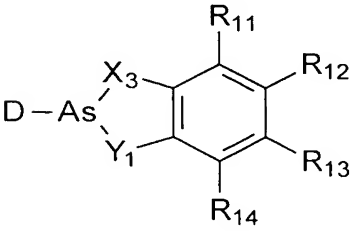


wherein X_3=NH, Y_1=O; X_3=Y_1=O or X_3=S, Y_1=O, and R' is selected from hydrogen, C_1-C_{10} alkyl, C_6-C_{12} aryl, halogen, C_1-C_{10} alkoxy, carboxy or isone of the twenty amino acid side chains;





wherein X_3=Y_1=O; X_3=NH, Y_1=O; X_3=S, Y_1=O; X_3=Y_1=NH; or X_3=S, Y_1=NH; or X_3 = S, Y_1 = NH and R_{11} to R_{14} are selected from hydrogen, C_1-C_{10} alkyl, C_6-C_{12} aryl, halogen, C_1-C_{10} alkoxy or CO_2H



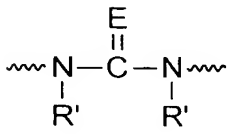
wherein X_3 =Y_1 = O, or X_3 = NH, Y_1 = O; and R_{11} to R_{14} are selected from hydrogen, C_1-C_{10} alkyl, C_6-C_{12} aryl, halogen, C_1-C_{10} alkoxy and CO_2H.

where D = GS-((XBX')$_n$B'), where (XBX')$_n$B' is any suitable spacer/linker group.

Typically X is selected from -NR-, -S(O)-, -S(O)O-, -S(O)$_2$-, -S(O)$_2$O-, -C(O)-, -C(S)-, -C(O)O-, C(S)O-, -C(S)S-, -P(O)(R_1)-, -P(O)(R_1)O- or is absent;

B is selected from C_1-C_{10} alkylene, C_2-C_{10} alkenylene, C_2-C_{10} alkynylene, C_3-C_{10} cycloalkylene, C_5-C_{10} cycloalkenylene, C_3-C_{10} heterocycloalkylene, C_5-C_{10} heterocycloalkenylene, C_6-C_{12} arylene, heteroarylene or C_2-C_{10} acyl;

X' is selected from –O-, -S-, -NR-, -S-S-, -S(O)-, -OS(O)-, -OS(O)O-, -S(O)$_2$-, -OS(O)$_2$, -OS(O)$_2$O-, -P(O)(R_1)-, -OP(O)(R_1)-, -OP(O)(R_1)O-, -OP(O)(R_1)OP(O)(R_1)O-, -Se-,



or is absent; wherein E is O, S, Se, NR or N(R)$_2^+$;

n is 0, 1 or 2; and

B' is C_1-C_{10} alkylene, C_2-C_{10} alkenylene, C_2-C_{10} alkynylene, C_3-C_{10} cycloalkylene, C_5-C_{10} cycloalkenylene, C_3-C_{10} heterocycloalkylene, C_5-C_{10} heterocycloalkenylene, C_6-C_{12} arylene, heteroarylene or is absent; and wherein

each R is independently selected from hydrogen, C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_3-C_{10} heterocycloalkyl, C_5-C_{10} heterocycloalkenyl, C_6-C_{12} aryl, heteroaryl, OR_2 or C_2-C_{10} acyl;

R' is the same as R or two R' may be taken together with the nitrogen atoms to which they are attached to form a 5 or 6 membered saturated or unsaturated heterocyclic ring;

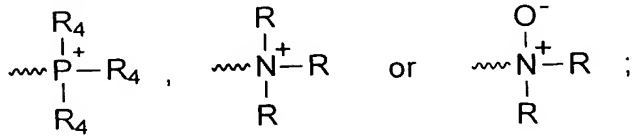
each R_1 is independently selected from hydrogen, C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_3-C_{10} heterocycloalkyl, C_5-C_{10} heterocycloalkenyl, C_6-C_{12} aryl, heteroaryl, halo, OR_2 or $N(R)_2$;

each R_2 is independently selected from hydrogen, C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_3-C_{10} heterocycloalkyl, C_5-C_{10} heterocycloalkenyl, C_6-C_{12} aryl, heteroaryl or -$C(O)R_5$;

each R_5 is independently selected from hydrogen, C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_3-C_{10} heterocycloalkyl, C_5-C_{10} heterocycloalkenyl, C_6-C_{12} aryl, heteroaryl, C_1-C_{10} alkoxy, C_3-C_{10} alkenyloxy, C_3-C_{10} alkynyloxy, C_3-C_{10} cycloalkyloxy, C_5-C_{10} cycloalkenyloxy, C_3-C_{10} heterocycloalkyloxy, C_5-C_{10} heterocycloalkenyloxy, C_6-C_{12} aryloxy, heteroaryloxy, C_1-C_{10} alkylthio, C_3-C_{10} alkenylthio, C_3-C_{10} alkynylthio, C_3-C_{10} cycloalkylthio, C_5-C_{10} cycloalkenylthio, C_3-C_{10} heterocycloalkylthio, C_5-C_{10} heterocycloalkenylthio, C_6-C_{12} arylthio, heteroarylthio, OH, SH or $N(R)_2$;

wherein each instance of arylene may have substituents A and X or X and Y in a para, meta or ortho relationship, and

wherein each alkylene, alkenylene, alkynylene, cycloalkylene, cycloalkenylene, heterocycloalkylene, heterocycloalkenylene, arylene, heteroarylene and acyl may be independently substituted with hydrogen, C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_3-C_{10} heterocycloalkyl, C_5-C_{10} heterocycloalkenyl, C_6-C_{12} aryl, heteroaryl, halo, OR_{2a}, SR_6, nitro, arsenoxide, -$S(O)R_3$, -$OS(O)R_3$, -$S(O)_2R_3$, -$OS(O)_2R_3$, -$P(O)R_4R_4$, -$OP(O)R_4R_4$, -$N(R'')_2$, -$NRC(O)(CH_2)_mQ$, -$C(O)R_5$,



wherein R, R_1 and R_5 are as defined above; and

R_{2a} is selected from hydrogen, C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_3-C_{10} heterocycloalkyl, C_5-C_{10} heterocycloalkenyl, C_6-C_{12} aryl, heteroaryl, -$S(O)R_3$, -$S(O)_2R_3$, -$P(O)(R_4)_2$, $N(R)_2$ or -$C(O)R_5$;

each R_3 is independently selected from hydrogen, C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_3-C_{10} heterocycloalkyl, C_5-C_{10} heterocycloalkenyl, C_6-C_{12} aryl, heteroaryl, C_1-C_{10} alkoxy, C_3-C_{10} alkenyloxy, C_3-C_{10} alkynyloxy, C_3-C_{10} cycloalkyloxy, C_5-C_{10}

cycloalkenyloxy, C_3-C_{10} heterocycloalkyloxy, C_5-C_{10} heterocycloalkenyloxy, C_6-C_{12} aryloxy, heteroaryloxy, C_1-C_{10} alkylthio, C_3-C_{10} alkenylthio, C_3-C_{10} alkynylthio, C_3-C_{10} cycloalkylthio, C_5-C_{10} cycloalkenylthio, C_3-C_{10} heterocycloalkylthio, C_5-C_{10} heterocycloalkenylthio, C_6-C_{12} arylthio, heteroarylthio or $N(R)_2$;

each R_4 is independently selected from hydrogen, C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_3-C_{10} heterocycloalkyl, C_5-C_{10} heterocycloalkenyl, C_6-C_{12} aryl, heteroaryl, C_1-C_{10} alkoxy, C_3-C_{10} alkenyloxy, C_3-C_{10} alkynyloxy, C_3-C_{10} cycloalkyloxy, C_5-C_{10} cycloalkenyloxy, C_3-C_{10} heterocycloalkyloxy, C_5-C_{10} heterocycloalkenyloxy, C_6-C_{12} aryloxy, heteroaryloxy, C_1-C_{10} alkylthio, C_3-C_{10} alkenylthio, C_3-C_{10} alkynylthio, C_3-C_{10} cycloalkylthio, C_5-C_{10} cycloalkenylthio, C_3-C_{10} heterocycloalkylthio, C_5-C_{10} heterocycloalkenylthio, C_6-C_{12} arylthio, heteroarylthio, halo or $N(R)_2$;

R_6 is selected from C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_3-C_{10} heterocycloalkyl, C_5-C_{10} heterocycloalkenyl, C_6-C_{12} aryl, heteroaryl, C_1-C_{10} alkylthio, C_3-C_{10} alkenylthio, C_3-C_{10} alkynylthio, C_3-C_{10} cycloalkylthio, C_5-C_{10} cycloalkenylthio, C_3-C_{10} heterocycloalkylthio, C_5-C_{10} heterocycloalkenylthio, C_6-C_{12} arylthio, heteroarylthio, $-S(O)R_3$, $-S(O)_2R_3$ or $-C(O)R_5$,

R" is the same as R or two R" taken together with the N atom to which they are attached may form a saturated, unsaturated or aromatic heterocyclic ring system;

Q is selected from halogen and $-OS(O)_2Q_1$; wherein Q_1 is selected from C_1-C_4 alkyl, C_1-C_4 perfluoroalkyl, phenyl, *p*-methylphenyl; and

m is 1 to 5.

Amino acid side chains are known to those of skill in the art and are listed, for instance in standard reference texts, such as King and Stansfield, A Dictionary of Genetics, 4th Edition, Oxford University Press, 1990, the contents of which are incorporated herein by reference.

The following features relate to the first and second embodiments of the invention.

Typically, the compound of the invention is a cell-membrane impermeable compound.

Typically, the compound of the invention uses glutathione as a cell membrane impermeable inert carrier of the reactive moiety which is an inhibitor of redox active proteins and which is linked to the glutathione portion of the inventive compound.

More typically, the compound of the invention is a cell-membrane impermeable dithiol-reactive compound.

The compounds of the invention may be linked to detector groups.

Typically, the detector group may be a chemical group, such as biotin.

Alternatively, the detector group is a radionucleide, such as ^{3}H, ^{14}C, ^{32}P, ^{33}P, ^{35}S, ^{125}I, ^{131}I, ^{123}I, ^{111}In, ^{105}Rh, ^{153}Sm, ^{67}Cu, ^{67}Ga, ^{166}Ho, ^{177}Lu, ^{186}Re, ^{188}Re, and ^{99m}Tc.

More typically, the radionucleide detector group is ^{3}H or ^{14}C.

According to a third embodiment of the invention, there is provided a process for preparing a compound of any one of the first or second embodiments of the invention, wherein said process comprises reacting a suitable multiply charged cell-membrane impermeable group (A) with a suitable linker and/or spacer group $(XBX')_nB'$ and at least one chemical moiety having the ability to inhibit

redox active proteins (Y), under conditions favourable to covalent bonding. A person skilled in the art will recognise that the specific order of reactions will be dependent on the particular compound of the invention which is being produced.

Typically, the process comprises reacting glutathione with a suitable linker and/or spacer group $(XBX')_nB'$ and at least one chemical moiety having the ability to inhibit redox active proteins (Y), under conditions favourable to the covalent bonding of said chemical moiety to said glutathione.

Typically, the reactive chemical moiety having the ability to inhibit redox active proteins comprises arsenoxide.

More typically, the reactive chemical moiety is phenylarsenoxide.

According to a fourth embodiment of the invention, there is provided a pharmaceutical composition comprising a compound of either of the first or second embodiments, together with a pharmaceutically acceptable carrier, adjuvant and/or diluent.

According to a fifth embodiment of the invention, there is provided a process for preparing a pharmaceutical composition according to the fourth embodiment, wherein said process comprises mixing a compound as defined in either of the first or second embodiments of the invention with a pharmaceutically acceptable carrier, adjuvant and/or diluent.

According to a sixth embodiment of the invention, there is provided a method of treatment and/or prophylaxis of disease in a vertebrate in need of said treatment and/or prophylaxis, wherein said method comprises administering to the vertebrate a therapeutically effective amount of the compound as defined in either of the first or second embodiments of the invention or administering a therapeutically effective amount of the pharmaceutical composition as defined in the fourth embodiment of the invention.

According to a seventh embodiment of the invention, there is provided the compound as defined in either of the first or second embodiments of the invention, or the pharmaceutical composition as defined in the fourth embodiment, when used in the treatment and/or prophylaxis of disease in a vertebrate in need of said treatment and/or prophylaxis.

According to an eighth embodiment of the invention, there is provided use of the compound as defined in either of the first or second embodiments of the invention, or of the pharmaceutical composition as defined in the fourth embodiment, in the preparation of a medicament for the treatment and/or prophylaxis of disease in a vertebrate in need of said treatment and/or prophylaxis.

Typically, in the sixth, seventh, or eighth embodiments of the invention, salts of the compounds of the present invention will be pharmaceutically acceptable salts; although other salts may be used in the preparation of the compound of the present invention or of the pharmaceutically acceptable salt thereof.

Typically, for the purposes of any one of the sixth, seventh, or eighth embodiments of the invention, the disease is a cellular proliferative disease.

More typically, the disease is selected from the group consisting of angiogenesis-dependent diseases, inflammatory disorders and/or auto-immune diseases, vascular disease and thrombosis, viral infection, and cancer.

Typically, for the purposes of any one of the sixth, seventh, or eighth embodiments of the invention, one skilled in the art would be able, by routine experimentation, to determine what an effective, non-toxic amount of the compound of the present invention would be for the purpose of treating the disease.

According to a ninth embodiment of the invention there is provided an assay for trivalent arsenicals.

Typically, the assay for trivalent arsenicals depends upon the ability of trivalent arsenicals to bind tightly to vicinal dithiols such as 2,3-dimercaptopropanol (DMP), and for free thiols to be detected photometrically using Ellman's reagent 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB). The active concentration of trivalent arsenical is found by titrating the arsenical with DMP, and determining the amount of free thiol (and hence of excess DMP) with DTNB. Specifically, increasing concentrations of the arsenical are prepared, and to each is added a constant amount of DMP such that there is more DMP than the smallest amount of arsenical but less DMP than the largest. Those with an excess of DMP will turn yellow with DTNB, and the actual concentration of DMP determined by measuring the absorbance at 412nm. Those with excess arsenical will not turn yellow as all thiol will be bound to As. Plotting the results gives the equivalence point (where the concentration of DMP = concentration of As), and as the initial concentration of DMP added to all samples is known, the active concentration of As is determined.

In a preferred embodiment the assay provides a method for determining the active concentration of arsenoxide.

Definitions

In the context of this specification, the term "comprising" means "including principally, but not necessarily solely". Furthermore, variations of the word "comprising", such as "comprise" and "comprises", have correspondingly varied meanings.

In the context of this specification, the terms "arsenoxide" and "arsenoso" refer to the same group, that is, the group –As=O.

In the context of this specification, the term "arsenoxide equivalent" means any trivalent arsenical that is (a) either hydrolysed to –As=O or $-As(OH)_2$ when dissolved in an aqueous medium (such as cell culture buffers and the fluids contained in the organism being treated), or (b) shows the same affinity towards dithiols as do –As=O and $-As(OH)_2$.

The term "alkyl" as used herein, includes within its meaning monovalent, saturated, straight and branched chain hydrocarbon radicals.

The term "alkenyl" as used herein, includes within its meaning, monovalent, straight and branched chain hydrocarbon radicals having at least one double bond.

The term "alkynyl" as used herein, includes within its meaning, monovalent, straight and branched chain hydrocarbon radicals having at least one triple bond.

The term "alkylene" as used herein, includes within its meaning divalent, saturated, straight chain hydrocarbon radicals.

The term "alkenylene" as used herein, includes within its meaning, divalent, straight chain hydrocarbon radicals having at least one double bond.

The term "alkynylene" as used herein, includes within its meaning, divalent, straight chain hydrocarbon radicals having at least one triple bond.

The term "cycloalkyl" as used herein, includes within its meaning monovalent, saturated, monocyclic, bicyclic, polycyclic or fused polycyclic hydrocarbon radicals.

The term "cycloalkylene" as used herein, includes within its meaning divalent, saturated, monocyclic, bicyclic, polycyclic or fused polycyclic hydrocarbon radicals.

The term "cycloalkenyl" as used herein, includes within its meaning monovalent, saturated, monocyclic, bicyclic, polycyclic or fused polycyclic hydrocarbon radicals having at least one double bond.

The term "cycloalkenylene" as used herein, includes within its meaning divalent, saturated, monocyclic, bicyclic, polycyclic or fused polycyclic hydrocarbon radicals having at least one double bond.

The term "heterocycloalkyl" as used herein, includes within its meaning monovalent, saturated, monocyclic, bicyclic, polycyclic or fused radicals wherein 1 to 5 atoms are heteroatoms selected from O, N or S.

The term "heterocycloalkylene" as used herein, includes within its meaning divalent, saturated, monocyclic, bicyclic, polycyclic or fused polycyclic radicals wherein 1 to 5 atoms are heteroatoms selected from O, N or S.

The term "heterocycloalkenyl" as used herein, includes within its meaning monovalent, saturated, monocyclic, bicyclic, polycyclic or fused polycyclic radicals having at least 1 double bond and wherein 1 to 5 atoms are heteroatoms selected from O, N or S.

The term "heterocycloalkenylene" as used herein, includes within its meaning divalent, saturated, monocyclic, bicyclic, polycyclic or fused polycyclic radicals having at least one double bond and wherein 1 to 5 atoms are heteroatoms selected from O, N or S.

The term "aryl" as used herein, includes within its meaning monovalent, single, polynuclear, conjugated and fused aromatic hydrocarbon radicals.

The term "arylene" as used herein, includes within its meaning divalent, single, polynuclear, conjugated and fused aromatic hydrocarbon radicals.

The term "heteroaryl" as used herein, includes within its meaning monovalent, single, polynuclear, conjugated and fused aromatic radicals having 1 to 12 atoms wherein 1 to 6 atoms are heteroatoms selected from O, N and S.

The term "heteroarylene" as used herein, includes within its meaning divalent, single, polynuclear, conjugated and fused aromatic radicals having 1 to 12 atoms wherein 1 to 6 atoms are heteroatoms selected from O, N and S.

The term "acyl" as used herein, includes monovalent and divalent alkyl, alkenyl, alkynyl, cycloalkyl and cycloalkenyl moieties possessing a terminal carbonyl substituent wherein attachment may occur at the hydrocarbon moiety, the carbonyl moiety or both.

The term "halo" as used herein, includes fluoro, chloro, bromo and iodo.

Abbreviations

p-arsanilic acid, 4-aminobenzenearsonic acid; DMP, 2,3-dimercaptopropanol; BRAA, 4-(*N*-(bromoacetyl)amino)phenylarsonic acid; BRAO.xH_2O, 4-(*N*-(bromoacetyl)amino)phenylarsenoxide hydrate; DMSO, dimethylsulphoxide; DTNB, 5,5'-dithiobis(2-nitrobenzoic acid); GSAA, 4-(*N*-(*S*-glutathionylacetyl)amino)phenylarsonic acid; GSAO, 4-(*N*-(*S*-glutathionylacetyl)amino)phenylarsenoxide; GSH, glutathione; H_4EDTA, ethylenediaminetetraacetic acid; HEPES, *N*-(2-hydroxyethyl)piperazine-*N'*-(2-ethanesulphonic acid); TNB, 5-thio-2-nitrobenzoate dianion.

Brief Description of the Drawings

Figure 1. Schematic representation of the synthesis of 4-(*N*-(bromoacetyl)amino)phenylarsonic acid and 4-(*N*-(bromoacetyl)amino)phenylarsenoxide hydrate.

Figure 2. Schematic representation of the synthesis of 4-(*N*-(*S*-glutathionylacetyl)amino)phenylarsenoxide.

Figure 3. Schematic representation of the reaction of GSAO with DMP.

Figure 4. Titration of GSAO with DMP. Varying concentrations of GSAO (0 to 41 μM) were incubated with DMP (15 μM) for 10 minutes at room temperature in 0.1 M Hepes, 0.3 M NaCl, pH 7.0 buffer. The reactions were incubated with DTNB (250 μM) for 10 minutes at room temperature and the absorbance at 405 nM was measured. The data is expressed as the SH concentration (2 X DMP) as a function of the GSAO/DMP ratio. The dotted line represents a 1:1 stoichiometry between GSAO and DMP.

Figure 5. Inhibition of thioredoxin by GSAO. Thioredoxin (1 μM) was incubated with GSAO (0, 1 or 10 μM) for 10 minutes at room temperature in 20 mM Hepes, 0.14 M NaCl, pH 7.4 buffer. The 70 kDa fibronectin fragment (10 μg per ml) was added and incubated for 5 minutes at room temperature. The reactions were labelled with MPB (100 μM) for 10 minutes at 37°C. The MPB was quenched with GSH (200 μM) for 10 minutes at 37°C followed by iodoacetamide (400 μM) for 10 minutes at room temperature. The MPB-labelled 70 kDa fragment was resolved on 5-15% SDS-PAGE, transferred to PVDF membrane and the MPB detected by blotting with streptavidin peroxidase. The positions of Mr markers are shown at left.

Figure 6. **A** Effect of GSAO on human dermal microvascular endothelial cell (HDMVEC) tube formation in Matrigel. HDMVEC were plated at 40,000 cells per well in 96 well plates containing 100 μl of Matrigel. In some cases the growth medium was supplemented with 1 μM GSAO. Phase contrast micrographs were taken at 7 and 24 hours. **B** Effect of GSAO on HDMVEC viability. HDMVEC were plated at 800 cells per well in 96 well plates on day 1. The growth medium was changed after 24 hours and supplemented with 0 to 1000 μM GSH (o) or GSAO (•). Viable cells were measured after a further 24 hours using Trypan Blue (12 and references therein). The values and error bars represent the mean and SE of triplicate determinations.

Figure 7. Schematic representation of the irreversible inhibition of a redox active protein by initial binding of an arsenoxide group with a dithiol of the protein, followed by alkylation of the active site.

Best Mode of Performing the Invention

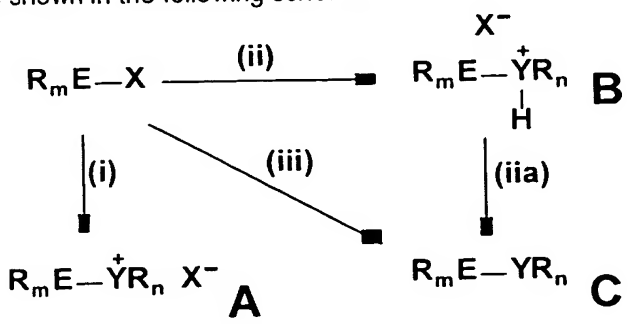
The present invention provides compounds wherein a chemical moiety having the ability to inhibit redox active proteins is linked to a cell membrane impermeable pendant group, that pendant group being cell membrane impermeable by virtue of being charged at neutral pH. That is, the present invention provides compounds in which an ionic pendant is linked, with or without the incorporation of a spacer group, to a chemical moiety having the ability to inhibit redox active proteins.

As a specific example of a cell membrane impermeable group which constitutes a suitable pendant group for the purposes of the present invention, glutathione is a tripeptide that is constitutively secreted by mammalian cells but is not taken up by these cells. In a preferred embodiment, the present invention capitalises on this cell-membrane impermeability feature of glutathione to use glutathione as an essentially inert carrier of a chemical moiety having the ability to inhibit redox active proteins. In this manner, glutathione is used in the present invention to deliver the reactive chemical moiety having the ability to inhibit redox active proteins to the mammalian cell surface, but to inhibit passive entry of said moiety into cells.

In a preferred form, the compound of the present invention is a dithiol reactive compound, such as a compound which contains a trivalent arsenical. Redox active proteins are often characterised by one or more pairs of closely spaced dithiols which undergo cycles of oxidation and reduction. Trivalent arsenicals have high affinity for closely spaced dithiols forming the dithioarsine derivatives (17). Monothiols react very poorly with trivalent arsenicals because two monothiols are required to form the dithioarsine derivatives. The binding of the second monothiol is usually sterically restricted.

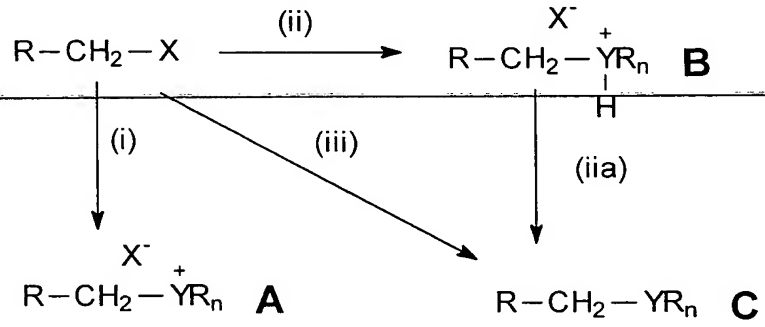
The compounds of general formula (I-VII) and those in which the arsenoso group (As=O) is replaced by an arsenoxide equivalent, may be prepared by methods known generally in the art. Suitable methods for the synthesis of compounds of formula (I-VII) and intermediates thereof are described, for example, in Houben-Weyl, *Methoden der Organischen Chemie;* J. March, *Advanced Organic Chemistry*, 4th Edition (John Wiley & Sons, New York, 1992); D. C. Liotta and M. Volmer, eds, *Organic Syntheses Reaction Guide* (John Wiley & Sons, Inc., New York, 1991); R. C. Larock, *Comprehensive Organic Transformations* (VCH, New York, 1989), H. O. House, *Modern Synthetic Reactions* 2nd Edition (W. A. Benjamin, Inc., Menlo Park, 1972); N. S. Simpkins, ed., *100 Modern Reagents* (The Royal Society of Chemistry, London, 1989); A. H. Hains *Methods for the Oxidation of Organic Compounds* (Academic Press, London, 1988) and B. J. Wakefield *Organolithium Methods* (Academic Press, London, 1988).

Example reaction schemes to illustrate the generic formation of linkers of the compounds of the present invention are shown in the following schemes.



where m, n are integers greater than or equal to 0.

The scheme below shows a starting molecule RCH_2X, where R represents the rest of the molecule to which the $-CH_2X$ group is attached. The X group (leaving group), which is usually halogen, but may also be RSO_3, is replaced by Y (nucleophile). Nucleophiles react with electrophiles, with the formation of a new covalent bond between the nucleophile and the electrophile. In this scheme, the methylene carbon atom is the electrophile, and the overall reaction can be described as one of nucleophilic substitution.



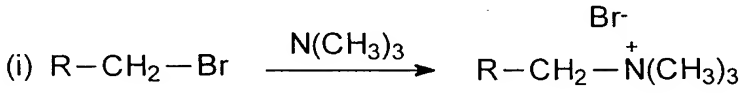
There are three simple variations on this scheme, as shown by reactions (i) to (iii):

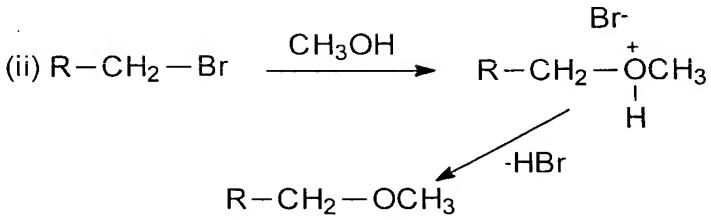
(i) in the first reaction, the attacking nucleophile is the uncharged molecule YR_n, which displaces X (coming off as X^-), the product **A** having a positive charge localised on Y.

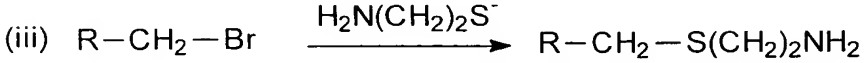
(ii) the second reaction has HYR_n as the attacking nucleophile, forming the analogous ionic product **B** initially, followed by loss of H^+ (reaction iia) to give the uncharged product **C**.

(iii) thirdly, product **C** can be formed directly by use of YR_n^-.

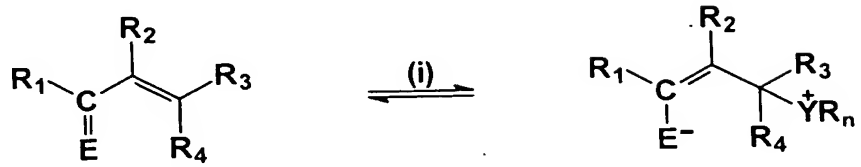
In all three reactions, X is lost as X^-, and atom Y must have a lone pair of electrons. Shown below are examples of each of the modifications (i) to (iii). Note that in (iii) the reaction corresponds to the formation of GSAO from BRAO and GSH.



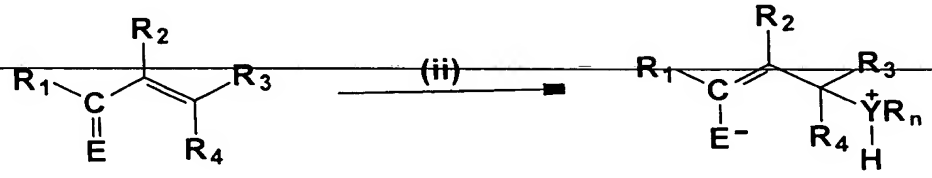


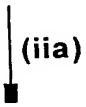


Alternatively, the coupling may be between a nucleophile and (when E=O) an α,β-unsaturated ketone (or aldehyde when R_1 = H) as illustrated in the following schemes. For example, where the nucleophile = YR_n

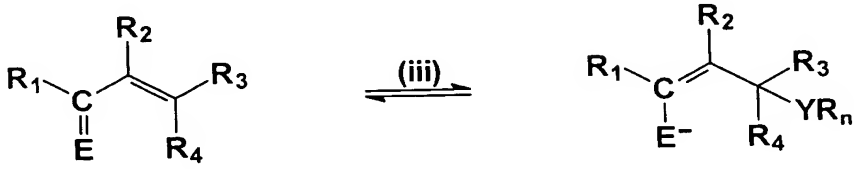


where the nucleophile = HYR_n





where the nucleophile = YR_n



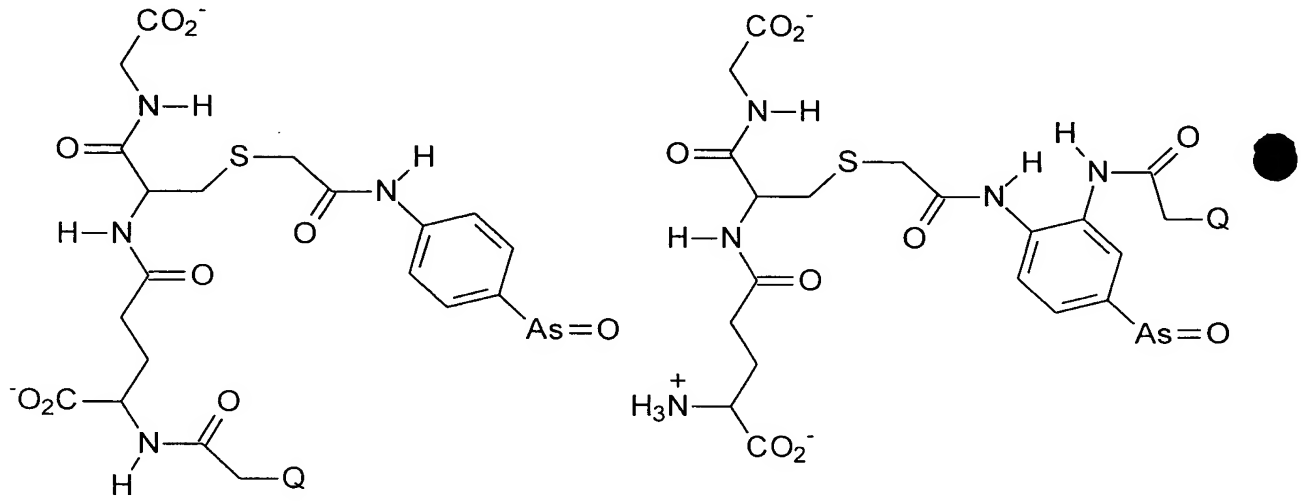
In a typical synthesis of compounds of the invention, glutathione may be reacted with the reactive chemical entity having the ability to inhibit redox active proteins under conditions favourable to the formation of covalent bonding of the reactive chemical entity to the free thiol of glutathione. Couplings involving nucleophilic attack of sulphur will in general require alkaline conditions. Electrophilic attack of some reactive species on the glutathione sulphur may be carried out; in general this would likely require acidic conditions. The compounds of the present invention can be lyophilised for storage and reconstituted prior to use.

A method of synthesis of GSAO is provided in Example 1.

Typically, the compounds of the invention are inhibitors of redox active proteins through an ability to bind dithiols and therein block redox cycling. Redox active proteins usually contain two closely spaced thiols which can reversibly form a disulphide bond. Trivalent arsenicals inhibit these proteins by binding to the reduced (dithiol) form of the protein. Such binding may be essentially irreversible or essentially reversible under physiological conditions. If the binding is essentially irreversible under physiological conditions, the protein is permanently inhibited from redox-cycling

between the dithiol and disulphide states (ie. it is irreversibly inactivated, or inhibited, by the arsenical).

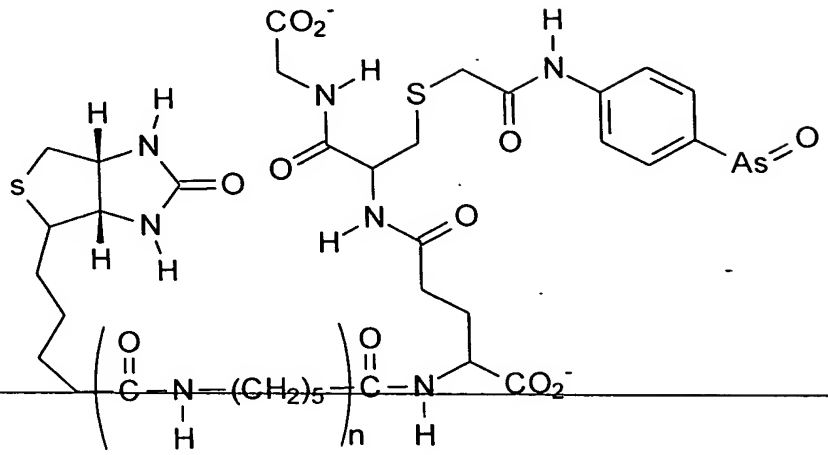
Alternatively, if the binding is essentially reversible under physiological conditions, inhibition will not be permanent and the present invention includes having a pendant group attached to the $(XBX')_nB'$ linker or the multiply charged, cell-membrane impermeable group, A, which can act as an alkylating agent. The pendant alkylating agent is brought into the vicinity of the active site of the protein by the reaction of the arsenical group with the dithiol of the protein and may then react with the protein to permanently inhibit redox-cycling. Compounds having an alkylating agent attached to the (XBX')nB' linker or the multiply charged, cell-membrane impermeable group, A, are exemplified by the following structural formulae:



wherein Q is a leaving group. An example of irreversible inhibition resulting from alkylation is shown in Figure 7. Any trivalent arsenical is expected to do this.

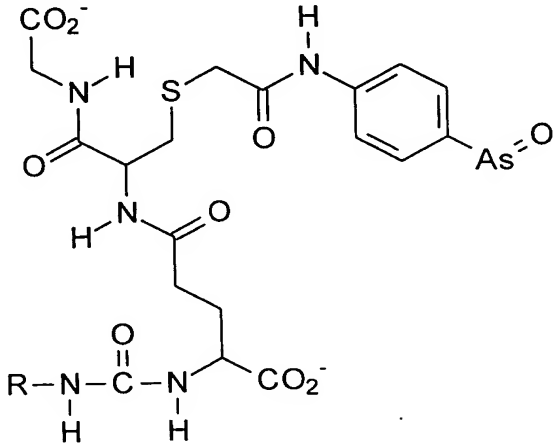
A person skilled in this art would recognise that the invention also provides for the compounds of the invention in any state of ionisation, for example acid salt, zwitterionic uncharged, zwitterionic anion, dianion.

The present invention also provides for a compound of the invention further modified through glutamyl α-amino nitrogen. Suitable modifications are known to those of skill in this art. For example, the invention provides for a biotin-linked derivative of GSAO, according to the following formula:



where n = 1 or 2.

Alternatively, a preferred linker or spacer group through which a desired modifying group may be attached through the glutamyl α-amino nitrogen is represented in the following formula:



wherein R is any desired modifying group.

Typically, R may be selected from the group consisting of: hydrogen, halogen, hydroxy, amino, nitro, carboxy, alkoxy, alkyl, and aryl.

The compounds of the invention are useful in the treatment of various disorders and diseases of vertebrates. Typically, the compounds of the first or second embodiments are useful in the treatment of various disorders and diseases of vertebrates. Also provided by the present invention therefore are methods of treatment of various diseases and disorders of vertebrates.

Typically, the vertebrate is selected from the group consisting of human, non-human primate, murine, bovine, ovine, equine, caprine, leporine, avian, feline and canine. More typically, the vertebrate is human, non-human primate or murine. Even more typically, the vertebrate is human.

Thus, the compounds of the invention may be useful for the treatment of disorders which may be grouped into broad categories such as the following: angiogenesis-dependent diseases, cellular proliferative diseases, inflammatory disorders, auto-immune diseases, blood vessel diseases, thrombosis, viral infection, and cancer. Typically, the diseases are those that depend on new blood vessel formation.

More typically, the compounds of the invention may be useful for the treatment of angiogenesis dependent diseases, such as solid tumors, hemangioma, arteriovenous malformations, arthritis, Osler-Weber Syndrome, complicated atherosclerotic plaques, psoriasis, corneal graft neovascularization, pyrogenic granuloma, delayed wound healing, retrolental fibroplasia, diabetic retinopathy, scleroderma, granulations, angiofibroma, neovascular glaucoma, trachoma, hemophilic joints, hypertrophic scars, gastric ulcers.

Examples of inflammatory disorders and/or auto-immune diseases are the following: rheumatoid arthritis, seronegative arthritides and other inflammatory arthritides, systemic lupus erythematosus, polyarteritis and related syndromes, systemic sclerosis, Sjögren's syndrome and other inflammatory eye disease, mixed connective tissue disease, polymyositis and dermatomyositis, polymyalgia rheumatica and giant cell arteritis, inflammatory joint disease, non-inflammatory arthropathies and soft tissue rheumatism, algodystrophy.

Examples of vascular disease and thrombosis for which the inventive compound may be used in a preventive manner or in the treatment of are the following: progression of atherosclerosis; cerebrovascular accidents such as transient ischaemic, completed stroke, and after carotid surgery; acute myocardial infarction (primary and secondary); angina; occlusion of coronary artery bypass graft; occlusion following percutaneous transluminal coronary angioplasty; occlusion following coronary stenting; vascular occlusion in peripheral arterial disease; venous thromboembolic disease following surgery, or during pregnancy, or during immobilisation.

Examples of small vessel disease for which the inventive compound may be used in prevention or treatment of are the following: glomerulonephritis; thrombotic thrombocytopenic purpura; the haemolytic uraemic syndrome; placental insufficiency and preeclampsia.

The compounds of the invention may also be used for the prevention or treatment of vascular syndromes and myeloproliferative diseases.

The compounds of the invention may also find use in the prevention of thrombosis formation in the following situations: artificial/prosthetic vascular shunts and grafts; prosthetic heart valves; cardiopulmonary bypass procedures; haemoperfusion and haemodialysis.

The compounds of the invention may also find use in the treatment or prevention of human retroviral infections (family retroviridae) including: oncoviral infection including HTLV-I; Lentiviral infection including HIV-1 and HIV-2; or for the treatment or prevention of Sindbis virus infection.

Typically, the cancer is selected from the group consisting of carcinogenic tumours, tumours of epithelial origin, such as colo-rectal cancer, breast cancer, lung cancer, head and neck tumours, hepatic cancer, pancreatic cancer, ovarian cancer, gastric cancer, brain cancer, bladder cancer, prostate cancer and urinary/genital tract cancer; mesenchymal tumours, such as sarcoma; and haemopoietic tumours such as B cell lymphoma.

Typically, the cancer is a haematological tumour.

More typically, the cancer is a solid tumour.

Typically, for medical use salts of the compounds of the present invention will be pharmaceutically acceptable salts; although other salts may be used in the preparation of the inventive compound or of the pharnaceutically acceptable salt thereof. By pharmaceutically

acceptable salt is meant those salts which are, within the scope of sound medical judgement, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art.

Suitable pharmaceutically acceptable salts of the compounds of the present invention may be prepared by mixing a pharmaceutically acceptable acid such as hydrochloric acid, sulphuric acid, methanesulphonic acid, succinic acid, fumaric acid, maleic acid, benzoic acid, phosphoric acid, acetic acid, oxalic acid, carbonic acid, tartaric acid, or citric acid. Suitable pharmaceutically acceptable salts of the compounds of the present invention therefore include acid addition salts.

For example, S. M. Berge *et al.* describe pharmaceutically acceptable salts in detail in J. *Pharmaceutical Sciences,* 1977, *66*:1-19. The salts can be prepared *in situ* during the final isolation and purification of the compounds of the invention, or separately by reacting the free base function with a suitable organic acid. Representative acid addition salts include acetate, adipate, alginate, ascorbate, asparate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphersulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptonate, glycerophosphate, hemisulfate, heptonate, hexanoate, hydrobromide, hydrochloride, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, toluenesulfonate, undecanoate, valerate salts, and the like. Representative alkali or alkaline earth metal salts include sodium, lithium potassium, calcium, magnesium, and the like, as well as nontoxic ammonium, quaternary ammonium, and amine cations, including, but not limited to ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, ethylamine, and the like.

One skilled in the art would be able, by routine experimentation, to determine an effective, non-toxic amount of the compound of the invention which would be required to treat or prevent the disorders and diseases to which the inventive compound is applicable. Generally, however, an effective dosage is expected to be in the range of about 0.0001 to about 100 mg GSAO per kg body weight per 24 hours, preferably about 0.001 to about 100 mg GSAO per kg body weight per 24 hours, more preferably about 0.01 mg to about 50 mg GSAO per kg body weight per 24 hours, even more preferably about 0.1 mg to about 20 mg GSAO per kg body weight per 24 hours, even more preferably still about 0.1 to about 10 mg GSAO per kg body weight per 24 hours. Typically the treatment would be for the duration of the condition. Contact times would typically be for the duration of the condition.

Also included within the scope of the present invention are prodrugs of the inventive compound. Typically, prodrugs will be functional derivatives of the compounds of the present invention which are readily converted *in vivo* to the required compound of the present invention as described herein. Typical procedures for the selection and preparation of prodrugs are known to those of skill in the art and are described, for instance, in H. Bundgaard (Ed), *Design of Prodrugs*, Elsevier, 1985.

When used in the treatment of disease the compounds of the present invention may be administered alone. However, it is generally preferable that the compound be administered as a pharmaceutical formulation. In general pharmaceutical formulations of the present invention may be prepared according to methods which are known to those of ordinary skill in the art and accordingly may include a pharmaceutically acceptable carrier, diluent and/or adjuvant.

The carriers, diluents and adjuvants must be "acceptable" in terms of being compatible with the other ingredients of the formulation, and not deleterious to the recipient thereof.

Examples of pharmaceutically and veterinarily acceptable carriers or diluents are demineralised or distilled water; saline solution; vegetable based oils such as peanut oil, safflower oil, olive oil, cottonseed oil, maize oil, sesame oils such as peanut oil, safflower oil, olive oil, cottonseed oil, maize oil, sesame oil, arachis oil or coconut oil; silicone oils, including polysiloxanes, such as methyl polysiloxane, phenyl polysiloxane and methylphenyl polysolpoxane; volatile silicones; mineral oils such as liquid paraffin, soft paraffin or squalane; cellulose derivatives such as methyl cellulose, ethyl cellulose, carboxymethylcellulose, sodium carboxymethylcellulose or hydroxypropylmethylcellulose; lower alkanols, for example ethanol or iso-propanol; lower aralkanols; lower polyalkylene glycols or lower alkylene glycols, for example polyethylene glycol, polypropylene glycol, ethylene glycol, propylene glycol, 1,3-butylene glycol or glycerin; fatty acid esters such as isopropyl palmitate, isopropyl myristate or ethyl oleate; polyvinylpyrridone; agar; carrageenan; gum tragacanth or gum acacia, and petroleum jelly. Typically, the carrier or carriers will form from 10% to 99.9% by weight of the compositions.

In a preferred form the pharmaceutical composition of the invention comprises an effective amount of the compound GSAO together with a pharmaceutically acceptable carrier, diluent and/or adjuvant a shown in Example 5.

The pharmaceutical composition of the invention may be in the form of a composition in a form suitable for administration by injection, in the form of a capsule suitable for oral ingestion, in the form of an ointment, cream or lotion suitable for topical administration, in a form suitable for delivery as an eye drop, in an aerosol form suitable for administration by inhalation, such as by intranasal inhalation or oral inhalation, in a form suitable for parenteral administration, that is, subcutaneous, intramuscular or intravenous injection.

For administration as an injectable solution or suspension, non-toxic parenterally acceptable diluents or carriers can include, Ringer's solution, isotonic saline, phosphate buffered saline, ethanol and 1,2 propylene glycol.

Some examples of suitable carriers, diluents, excipients and adjuvants for oral use include peanut oil, liquid paraffin, sodium carboxymethylcellulose, methylcellulose, sodium alginate, gum acacia, gum tragacanth, dextrose, sucrose, sorbitol, mannitol, gelatine and lecithin. In addition these oral formulations may contain suitable flavouring and colourings agents. When used in capsule form the capsules may be coated with compounds such as glyceryl monostearate or glyceryl distearate which delay disintegration.

Adjuvants typically include emollients, emulsifiers, thickening agents, preservatives, bactericides and buffering agents.

Solid forms for oral administration may contain binders acceptable in human and veterinary pharmaceutical practice, sweeteners, disintegrating agents, diluents, flavourings, coating agents, preservatives, lubricants and/or time delay agents. Suitable binders include gum acacia, gelatine, corn starch, gum tragacanth, sodium alginate, carboxymethylcellulose or polyethylene glycol. Suitable sweeteners include sucrose, lactose, glucose, aspartame or saccharine. Suitable disintegrating agents include corn starch, methylcellulose, polyvinylpyrrolidone, guar gum, xanthan gum, bentonite, alginic acid or agar. Suitable diluents include lactose, sorbitol, mannitol, dextrose, kaolin, cellulose, calcium carbonate, calcium silicate or dicalcium phosphate. Suitable flavouring agents include peppermint oil, oil of wintergreen, cherry, orange or raspberry flavouring. Suitable coating agents include polymers or copolymers of acrylic acid and/or methacrylic acid and/or their esters, waxes, fatty alcohols, zein, shellac or gluten. Suitable preservatives include sodium benzoate, vitamin E, alpha-tocopherol, ascorbic acid, methyl paraben, propyl paraben or sodium bisulphite. Suitable lubricants include magnesium stearate, stearic acid, sodium oleate, sodium chloride or talc. Suitable time delay agents include glyceryl monostearate or glyceryl distearate.

Liquid forms for oral administration may contain, in addition to the above agents, a liquid carrier. Suitable liquid carriers include water, oils such as olive oil, peanut oil, sesame oil, sunflower oil, safflower oil, arachis oil, coconut oil, liquid paraffin, ethylene glycol, propylene glycol, polyethylene glycol, ethanol, propanol, isopropanol, glycerol, fatty alcohols, triglycerides or mixtures thereof.

Suspensions for oral administration may further comprise dispersing agents and/or suspending agents. Suitable suspending agents include sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethyl-cellulose, poly-vinyl-pyrrolidone, sodium alginate or acetyl alcohol. Suitable dispersing agents include lecithin, polyoxyethylene esters of fatty acids such as stearic acid, polyoxyethylene sorbitol mono- or di-oleate, -stearate or -laurate, polyoxyethylene sorbitan mono- or di-oleate, -stearate or -laurate and the like.

The emulsions for oral administration may further comprise one or more emulsifying agents. Suitable emulsifying agents include dispersing agents as exemplified above or natural gums such as guar gum, gum acacia or gum tragacanth.

The topical formulations of the present invention, comprise an active ingredient together with one or more acceptable carriers, and optionally any other therapeutic ingredients.

Formulations suitable for topical administration include liquid or semi-liquid preparations suitable for penetration through the skin to the site of where treatment is required, such as liniments, lotions, creams, ointments or pastes, and drops suitable for administration to the eye, ear or nose.

Drops according to the present invention may comprise sterile aqueous or oily solutions or suspensions. These may be prepared by dissolving the active ingredient in an aqueous solution of a bactericidal and/or fungicidal agent and/or any other suitable preservative, and optionally including a surface active agent. The resulting solution may then be clarified by filtration, transferred to a suitable container and sterilised. Sterilisation may be achieved by: autoclaving or maintaining at 90°C-100°C for half an hour, or by filtration, followed by transfer to a container by an aseptic technique. Examples of bactericidal and fungicidal agents suitable for inclusion in the drops are phenylmercuric nitrate or

acetate (0.002%), benzalkonium chloride (0.01%) and chlorhexidine acetate (0.01%). Suitable solvents for the preparation of an oily solution include glycerol, diluted alcohol and propylene glycol.

Lotions according to the present invention include those suitable for application to the skin or eye. An eye lotion may comprise a sterile aqueous solution optionally containing a bactericide and may be prepared by methods similar to those described above in relation to the preparation of drops. Lotions or liniments for application to the skin may also include an agent to hasten drying and to cool the skin, such as an alcohol or acetone, and/or a moisturiser such as glycerol, or oil such as castor oil or arachis oil.

Creams, ointments or pastes according to the present invention are semi-solid formulations of the active ingredient for external application. They may be made by mixing the active ingredient in finely-divided or powdered form, alone or in solution or suspension in an aqueous or non-aqueous fluid, with a greasy or non-greasy basis. The basis may comprise hydrocarbons such as hard, soft or liquid paraffin, glycerol, beeswax, a metallic soap; a mucilage; an oil of natural origin such as almond, corn, arachis, castor or olive oil; wool fat or its derivatives, or a fatty acid such as stearic or oleic acid together with an alcohol such as propylene glycol or macrogols.

The formulation may incorporate any suitable surface active agent such as an anionic, cationic or non-ionic surface active such as sorbitan esters or polyoxyethylene derivatives thereof. Suspending agents such as natural gums, cellulose derivatives or inorganic materials such as silicaceous silicas, and other ingredients such as lanolin, may also be included.

Further, it will be apparent to one of ordinary skill in the art that the optimal quantity and spacing of individual dosages of a compound of the present invention will be determined by the nature and extent of the condition being treated, the form, route and site of administration, and the nature of the particular vertebrate being treated. Also, such optimum conditions can be determined by conventional techniques.

It will also be apparent to one of ordinary skill in the art that the optimal course of treatment, such as, the number of doses of the compound of the present invention given per day for a defined number of days, can be ascertained by those skilled in the art using conventional course of treatment determination tests.

The compositions for parenteral administration will commonly comprise a solution of a compound of the present invention or a cocktail thereof dissolved in an acceptable carrier, such as water, buffered water, 0.4% saline, and 0.3% glycine etc, wherein such solutions are sterile and relatively free of particulate matter. These solutions are then subsequently sterilised.

Methods for preparing parenterally administrable compositions are apparent to those skilled in the art, and are described in more detail in, for example, Remington's Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, Pa., hereby incorporated by reference herein.

The compositions may contain further pharmaceutically acceptable substances as required to approximate physiological conditions such as pH adjusting and buffering agents, etc. The concentration of the compound of the present invention in such a composition can vary, and will be primarily based on fluid volumes, viscosities, etc., according to the particular mode of administration selected.

Depending on the intended result, the pharmaceutical composition of the present invention can be administered for prophylactic and/or therapeutic treatments. In a therapeutic application, compositions are administered to a patient already suffering from a disease, in an amount sufficient to cure or at least partially arrest the disease and its complications. In prophylactic applications, compositions containing the compound or a cocktail thereof are administered to a patient not already in a disease state to enhance the patient's resistance.

Single or multiple administrations of the pharmaceutical compositions can be carried out with dose levels and pattern being selected by the treating physician. Regardless, the pharmaceutical composition of the present invention should provide a quantity of the compound sufficient to effectively treat the patient.

The invention will now be described in greater detail by reference to specific Examples, which should not be construed as in any way limiting the scope of the invention.

Examples

Experimental Procedures

Chemicals and proteins - The following chemicals were purchased and used without further purification: bromoacetyl bromide, sulphur dioxide, d_6-dimethylsulphoxide, deuterium oxide (Aldrich, Castle Hill, NSW); methanol, 98% sulphuric acid, 48% hydrobromic acid, 37% hydrochloric acid (Ajax, Auburn, NSW); dichloromethane, potassium hydroxide, sodium hydrogen carbonate, sodium hydroxide (BDH, Kilsyth, VIC); P-2 Gel extra fine 1,800 MW cut-off (Bio-Rad, Hercules, CA); 2,3-dimercaptopropanol (Fluka, Castle Hill, NSW); dimethylsulphoxide, 5,5'-dithiobis(2-nitrobenzoic acid), ethylenediaminetetraacetic acid, *N*-(2-hydroxyethyl)piperazine-*N*'-(2-ethanesulphonic acid), glutathione, sodium carbonate, sodium chloride, sodium iodide (Sigma, Castle Hill, NSW); *p*-arsanilic acid (Tokyo Kasei Kogyo, Tokyo, Japan). Recombinant *E. coli* thioredoxin was from Promega, Annandale, NSW, while the 70 kDa N-terminal fragment of fibronectin was from Sigma, Castle Hill, NSW.

Example 1

Synthesis of 4-(*N*-(*S*-glutathionylacetyl)amino)phenylarsenoxide (GSAO)

Synthesis of 4-(*N*-(bromoacetyl)amino)phenylarsonic acid (BRAA) - Into a stirred solution of potassium hydroxide (10.31 g, 184 mmol) in water (100 ml) was added *p*-arsanilic acid (20.39 g, 94 mmol), the flask swirled until the acid completely dissolved. Sodium carbonate (30.56 g, 288 mmol) and water (100 ml) were added, with stirring continued until all solids had dissolved to give a warm solution. Ice chips were added to the solution until it was cold enough not to melt the ice immediately. It was then poured into a 500 ml separating funnel, and half of a solution of bromoacetyl bromide (12 ml, 27.8 g, 138 mmol) in dichloromethane (50 ml) was cautiously added. The funnel was stoppered and shaken until production of gas had ceased before the remaining bromoacetyl bromide was added. This time, the mixture was shaken vigorously for about 5 minutes, by which time no more gas was evolved. The organic layer was drained off, and the aqueous layer (found to be pH 10) was transferred to a 500 ml conical flask. Careful acidification of the aqueous layer with 98% sulphuric acid to approximately pH 1 resulted in the formation of the product as a fine white precipitate, which

was collected and air-dried over night using a Büchner funnel, giving 24.55 g (73 mmol, 78% yield). ^{1}H-NMR (d_6-DMSO): δ 4.09 (s, 2H), 7.73 (d, J = 9 Hz, 2H), 7.83 (d, J = 9 Hz, 2H), 10.87 (s, 1H). ^{13}C-NMR (d_6-DMSO): δ 30.53, 119.97, 127.34, 131.56, 143.08, 166.00 ppm.

Synthesis of 4-(*N*-(bromoacetyl)amino)phenylarsenoxide hydrate (BRAO.xH_2O) - Into a 3-necked 500 ml round-bottomed flask was placed BRAA (12.15 g, 36 mmol). The solid was dissolved with swirling by the addition of methanol (75 ml) and 48% hydrobromic acid (75 ml), giving a transparent yellow solution. Any undissolved solid left at this point was removed by filtration. Sodium iodide (0.20 g, 1.3 mmol) was added as a catalyst, causing the colour of the solution to darken to an orange-brown, and sulphur dioxide gas was slowly (ca 2 bubbles per second) passed through the stirred solution for 2 hours and 20 minutes. The white precipitated product was collected using a Büchner funnel, giving 17.43 g of damp white solid. The activity of a solution made by dissolving 40.7 mg of solid in 800 µL of deoxygenated DMSO was determined to be 56 mM (see below). Hence, the molecular weight of BRAO.xH_2O is 908.5, that is 35% w/w BRAO and 65% w/w H_2O. Therefore, the "anhydrous" weight for BRAO is 35% of 17.43 g, that is 6.10 g (19 mmol, 53% yield). ^{1}H-NMR (d_6-DMSO): δ 4.85 (s, 2H), 7.78 (d, J = 9 Hz, 2H), 7.86 (d, J = 9 Hz, 2H), 11.36 (s, 1H). ^{13}C-NMR (d_6-DMSO): δ 30.55, 119.22, 130.52, 140.04, 145.04, 165.52 ppm.

Synthesis of 4-(*N*-(*S*-glutathionylacetyl)amino)phenylarsenoxide (GSAO) - DMSO (10 ml) was deoxygenated by passing a stream of nitrogen gas through it for a few minutes, and used to dissolve BRAO.xH_2O, 20% w/w H_2O (1.00 g, 2.48 mmol active arsenoxide). Glutathione (1.15 g, 3.74 mmol, 1.5 eq) was dissolved in 0.5 M bicarbonate buffer, pH 9.6 (35 ml), and added to the solution of BRAO.xH_2O in DMSO. The total volume was made up to 50 ml with 0.5 M bicarbonate buffer, and the solution gently agitated at room temperature for 3 hours. Cautious neutralisation with 37% hydrochloric acid, followed by lyophilisation, gave a white powdery product which was freely soluble in water with no solid residue. The active arsenoxide concentration of the solution was 49.6 mM (see below).

The product was purified using gel-filtration (P-2 Gel extra fine, 1.8 kDa cutoff, 50 g) with 20 mM Hepes, 0.14 M NaCl, 1 mM EDTA, pH 7.4 buffer as the eluant in a 130 ml column (V_t = 130 ml, V_0 = 43 ml, V_i = 87 ml; flow rate was 0.10 ml/min). A total of 144 ml was collected in 72 fractions of 2 ml, with the flow-through monitored by UV. The UV trace showed four main peaks: A (fractions 17 to 23, large absorbance), B (27 to 32, large absorbance), C (48 to 52, small absorbance) and D (56 to 60, small absorbance). Peaks B and C were found to show activity in the DTNB/DMP assay (see below), and were assigned as the desired GSAO and unreacted BRAO, respectively. Peaks A and D showed no activity, and are probably the oxidation products GSAA and BRAA, respectively. Peak A alone showed activity due to free thiol, and so all 72 fractions were tested against DTNB: the results indicated that the free thiol appeared only in fractions 18 to 22 (entirely within Peak A), and was assigned as unreacted glutathione. The fractions making up Peak B were combined and deoxygenated with nitrogen gas, giving about 12 ml of the active compound GSAO, the concentration determined as 15.5 mM. ^{1}H-NMR (D_2O): δ1.93 (q, J = 7 Hz, 2H), 2.35 (t, J = 8 Hz, 2H), 2.84 (dd, J = 14 Hz, J = 9 Hz, 1H), 3.05 (dd, J = 14 Hz, J = 5 Hz, 1H), 3.35 (s, 2H), 3.58 (t, J = 6 Hz, 1H), 3.64 (d, J = 2 Hz, 2H), 4.48 (dd, J = 9 Hz, J = 5 Hz, 1H), 7.44 (d, J = 8 Hz, 2H), 7.58 (d, J = 8 Hz, 2H). ^{13}C-NMR

(D_2O): δ 25.93, 31.16, 33.53, 36.01, 42.97, 52.83, 53.89, 121.29, 129.97, 138.77, 144.09, 170.90, 171.73, 173.75, 174.68, 175.76 ppm.

Example 2

Assay of GSAO

The basis for this assay is the formation of the yellow 5-thio-2-nitrobenzoate (TNB) dianion from the exchange reaction between a free thiol (in this case 2,3-dimercaptopropanol) and DTNB. Trivalent arsenicals are able to bind to vicinal dithiols (that is, dithiols where the two sulphur atoms are close enough to be able to form a cyclic complex with the arsenic atom). 2,3-Dimercaptopropanol (DMP) is a good example of a vicinal dithiol, and forms a very stable complex with GSAO. As thiols bound to arsenic in this way cannot react with DTNB, the active concentration of arsenoxide can be determined by the amount needed to completely prevent the formation of the yellow TNB dianion.

The solution containing the arsenoxide was incubated with DMP (~ 30 μM) in 0.1 M HEPES, 0.3 M NaCl, 1 mM EDTA, pH 7.0 buffer for 10 minutes at room temperature. DMP (5 μL, 50 μmol) was dissolved with DMSO (195 μL), giving a 0.25 M stock solution. DTNB (250 μM) was then added and incubated for 5 minutes at room temperature. DTNB (15 mg, 37.9 μmol) was dissolved in DMSO (1.00 ml) to give a 37.9 mM stock solution. The absorbance at 412 nm was measured using a Molecular Devices Thermomax Plus (Palo Alto, CA) microplate reader. The extinction coefficient for the TNB dianion at pH 7.0 is 14,150 $M^{-1}cm^{-1}$ at 412 nm (1).

Example 3

SDS-PAGE and Blotting

Samples were resolved on 5-15% SDS-PAGE under non-reducing conditions according to Laemmli (6), transferred to PVDF membrane, developed according to the manufacturers instructions (DuPont, Boston, MA), and visualized using chemiluminescence. Streptavidin-HRP was used at 1:2000 dilution.

Example 4

HDMVEC culture

HDMVEC were harvested and maintained as described previously (12).

Results and Discussion

GSAO was found to be a potent inhibitor of the 12 kDa redox active protein, thioredoxin (16). The ability of thioredoxin to catalyse disulfide reduction in proteins resides in a very reactive surface exposed dithiol/disulfide in the sequence WCGPCK, which has a redox potential of -235 mV. Thioredoxin is secreted by cells (17). Thioredoxin reduces one or more protein disulfide bonds in the 70 kDa N-terminal fragment of fibronectin. This property of thioredoxin was used to assay the inhibitory activity of GSAO. Incubation of 1 μM of GSAO with 1 μM of thioredoxin for 10 minutes in Hepes buffered saline resulted in ~50% inhibition of thioredoxin-mediated reduction of the fibronectin fragment, whereas incubation with 10 μM of GSAO completely inhibited thioredoxin activity (Figure 5). This finding implied that GSAO bound to the active site dithiol of thioredoxin with a dissociation constant of < 1 μM, and confirmed the inhibitory effect of GSAO on redox active proteins.

When endothelial cells are suspended in an extracellular matrix such as collagen gel or Matrigel, a basement membrane preparation from EHS mouse sarcoma cells, they migrate and organise into tubes that resemble immature blood vessels. GSAO inhibited HDMVEC tube formation in Matrigel (Figure 6A). Effects were observed at 0.1 μM GSAO (not shown) and were marked at 1 μM GSAO. It was apparent that the rate of formation of tubes slowed and the final tube structure was much less well defined. These effects of GSAO did not appear to be due to HDMVEC cell death as thousand-fold higher concentrations of GSAO were required to significantly effect HDMVEC viability (Figure 6B). GSAO may inhibit endothelial cell migration and tube formation through inactivation of one or more endothelial cell surface redox active proteins. These observations suggest that GSAO may be a useful therapeutic for the treatment of diseases that depend on new blood vessel formation such as rheumatoid arthritis and solid tumours.

GSAO is the first membrane-impermeable inhibitor of redox active protein dithiols. It has potential as a drug for the treatment of a broad spectrum of human disease. For instance, GSAO may be an effective drug for the treatment of inflammatory disorders such as rheumatoid arthritis, auto-immune diseases such as systemic lupus erythematosus, blood vessel diseases such as atherosclerosis, unwanted thrombosis such as associated with thrombotic thrombocytopenic purpura, viral infections such as AIDS, and haematological and solid tumours.

Example 5

The compound of the present invention may be administered alone, although it is preferable that it be administered as a pharmaceutical formulation. The active ingredient may comprise, for topical administration, from 0.001% to 10% by weight, eg., from 1% to 5% by weight of the formulation, although it may comprise as much as 10% by weight but preferably not in excess of 5% by weight, and more preferably from 0.1% to 1% by weight of the formulation.

In accordance with the detailed description of the invention provided herein specific preferred pharmaceutical compositions of the present invention are provided hereinafter. The following are to be construed as merely illustrative examples of formulations and not as a limitation of the scope of the present invention in any way.

Example 5(a) - Topical Cream Composition

A typical composition for delivery as a topical cream is outlined below:

GSAO 1.0 g
Polawax GP 200 25.0 g
Lanolin Anhydrous 3.0 g
White Beeswax 4.5 g
Methyl hydroxybenzoate 0.1 g
Deionised & sterilised Water to 100.0 g

The polawax, beeswax and lanolin are heated together at 60°C, a solution of methyl hydroxybenzoate is added and homogenisation is achieved using high speed stirring. The temperature is then allowed to fall to 50°C. The compound of the present invention, in this example being GSAO, is then added and dispersed throughout, and the composition is allowed to cool with slow speed stirring.

Example 5(b) - Topical Lotion Composition

A typical composition for delivery as a topical lotion is outlined below:

GSAO 1.2 g
Sorbitan Monolaurate 0.8 g
Polysorbate 20 0.7 g
Cetostearyl Alcohol 1.5 g
Glycerin 7.0 g
Methyl Hydroxybenzoate 0.4 g
Sterilised Water about to 100.00 ml

The methyl hydroxybenzoate and glycerin are dissolved in 70 ml of the water at 75°C. The sorbitan monolaurate, polysorbate 20 and cetostearyl alcohol are melted together at 75°C and added to the aqueous solution. The resulting emulsion is homogenised, allowed to cool with continuous stirring and the GSAO of the present invention is added as a suspension in the remaining water. The whole suspension is stirred until homogenised.

Example 5(c) - Eye Drop Composition

A typical composition for delivery as an eye drop is outlined below:

GSAO 0.3 g
Methyl Hydroxybenzoate 0.005 g
Propyl Hydroxybenzoate 0.06 g
Purified Water about to 100.00 ml.

The methyl and propyl hydroxybenzoates are dissolved in 70 ml purified water at 75°C, and the resulting solution is allowed to cool. The GSAO of the invention is then added, and the solution sterilised by filtration through a membrane filter (0.22 μm pore size), and aseptically packed into sterile containers.

Example 5(d) - Composition for Inhalation Administration

For an aerosol container with a capacity of 20-30 ml: a mixture of 10 mg of GSAO with 0.5-0.8% by weight of a lubricating agent, such as polysorbate 85 or oleic acid, and mixture was dispersed in a propellant, such as freon, and put into an appropriate aerosol container for either intranasal or oral inhalation administration.

Example 5(f) - Composition for Parenteral Administration

A pharmaceutical composition of the present invention for intramuscular injection could be prepared to contain 1 mL sterile buffered water, and 1 mg of GSAO.

Similarly, a pharmaceutical composition for intravenous infusion may comprise 250 ml of sterile Ringer's solution, and 5 mg of GSAO.

Example 5(g) - Capsule Composition

A pharmaceutical composition GSAO in the form of a capsule may be prepared by filling a standard two-piece hard gelatin capsule with 50 mg of GSAO, in powdered form, 100 mg of lactose, 35 mg of talc and 10 mg of magnesium stearate.

Example 5(h) - Injectable Parenteral Composition

A pharmaceutical composition of this invention in a form suitable for administration by injection may be prepared by mixing 1% by weight of GSAO in 10% by volume propylene glycol and water. The solution is sterilised by filtration.

Example 5(i) - Ointment Composition

A typical composition for delivery as an ointment includes 1.0g of GSAO, together with white soft paraffin to 100.0 g, is dispersed to produce a smooth, homogeneous product.

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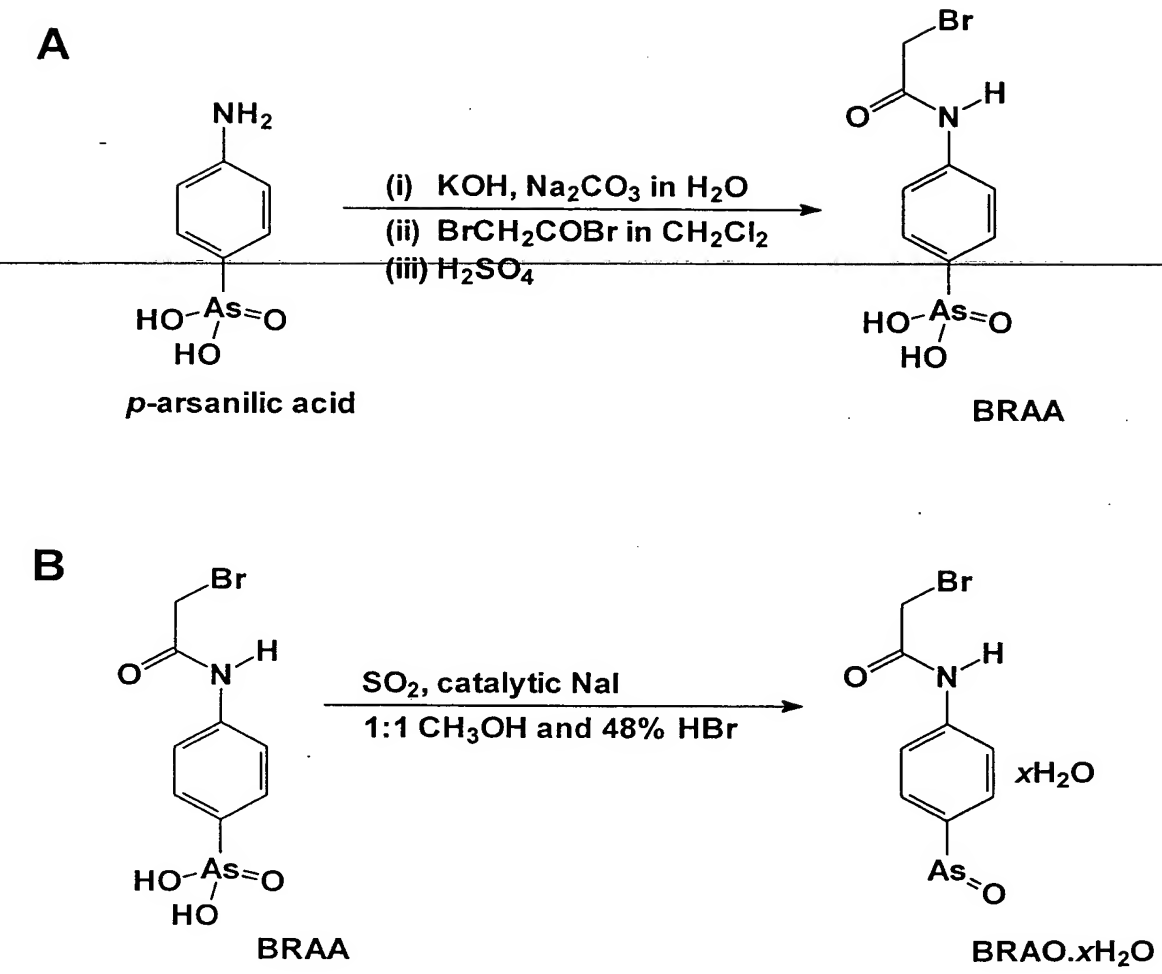
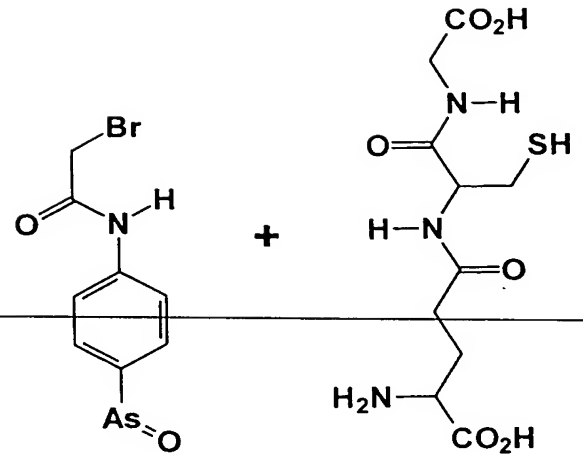
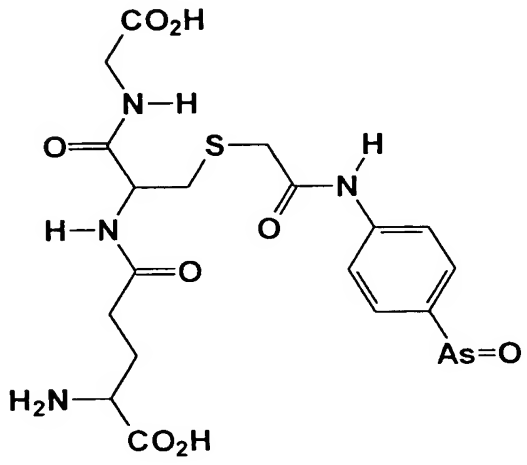

A
NH2
HO-As=O
HO
p-arsanilic acid
(i) KOH, Na2CO3 in H2O
(ii) BrCH2COBr in CH2Cl2
(iii) H2SO4
Br
O
N
H
HO-As=O
HO
BRAA
B
Br
O
N
H
HO-As=O
HO
BRAA
SO2, catalytic NaI
1:1 CH3OH and 48% HBr
Br
O
N
H
xH2O
As=O
BRAO.xH2O

FIGURE 1



BRAO glutathione (GSH)

pH 9.6



GSAO

FIGURE 2

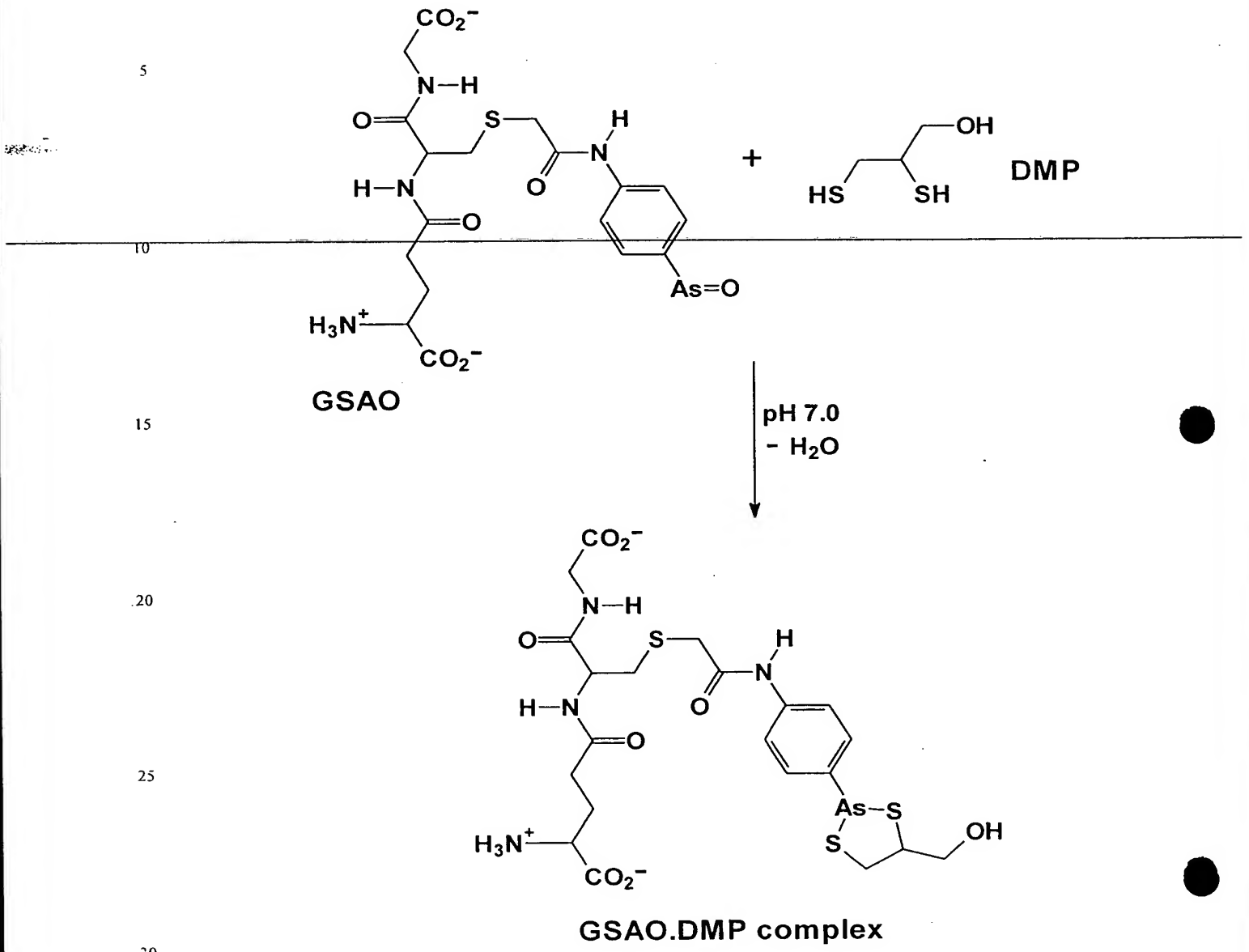


FIGURE 3

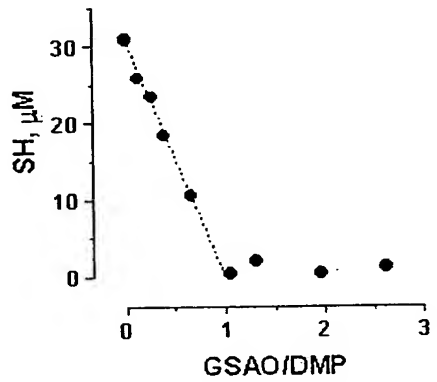


FIGURE 4

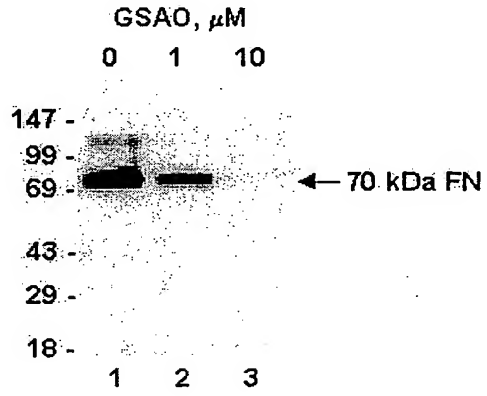
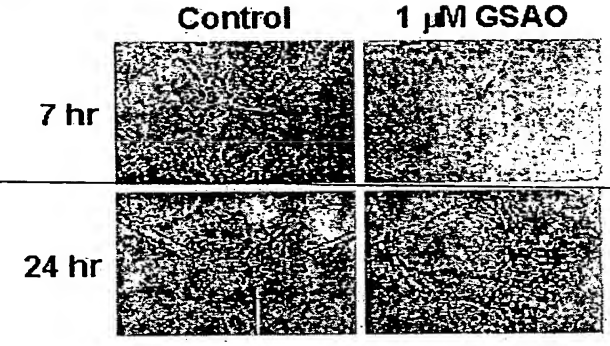


FIGURE 5

A



B

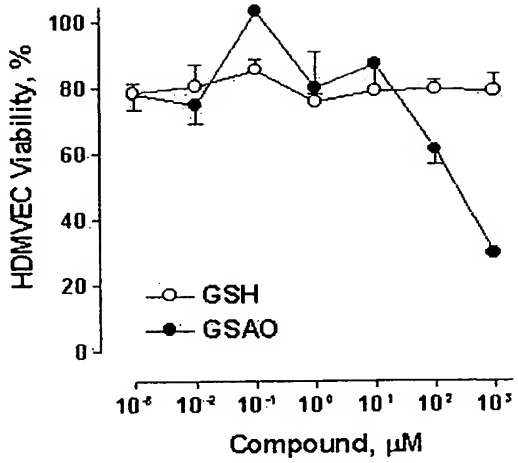


Figure 6A and B

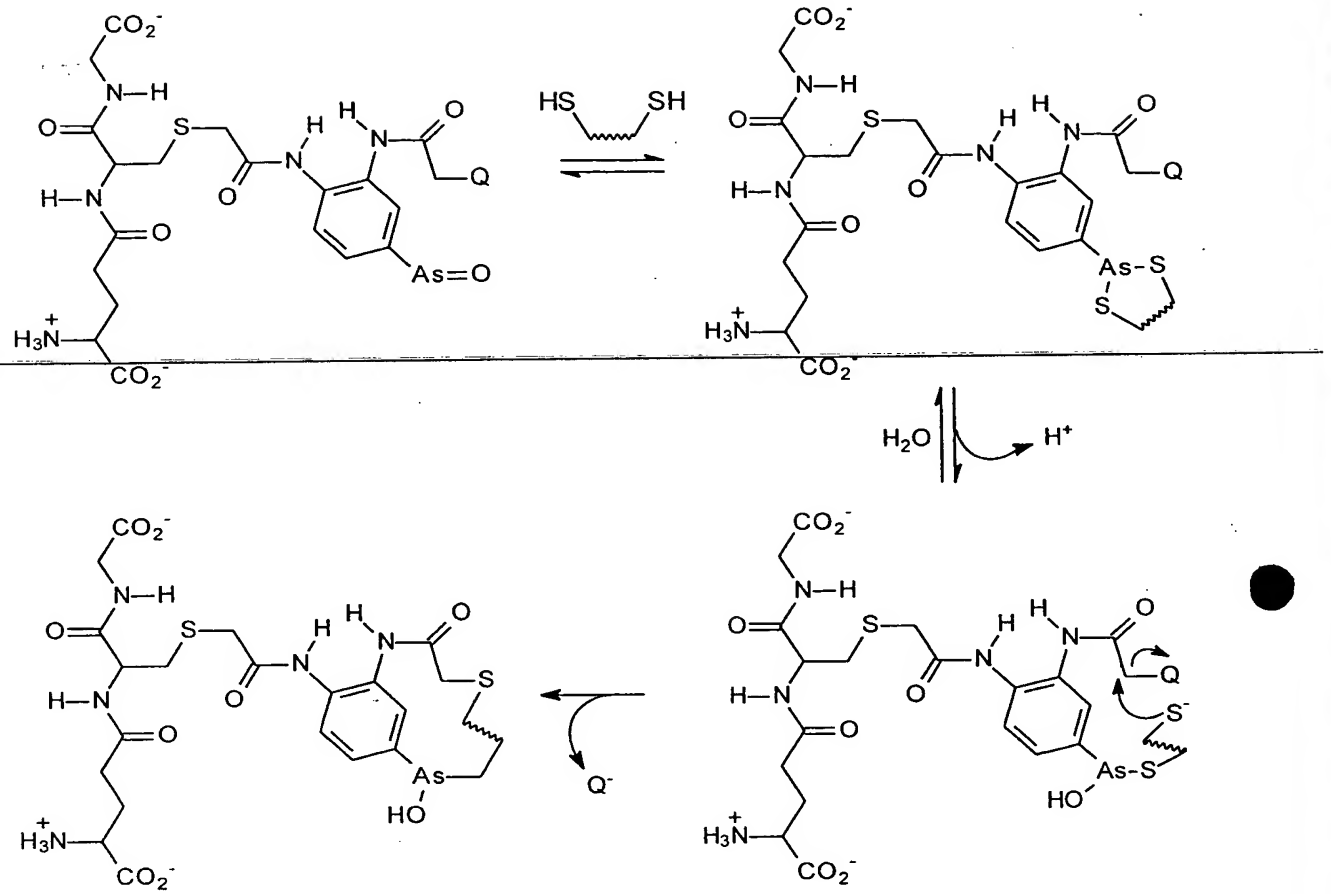


FIGURE 7